SIDRA Summer School – Systems Biology course

Notes on Biochemical Reaction Networks and Gene Circuits

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Contents

1 Introduction

These notes are inspired (when not copied) from various sources. For what concerns the theory of biochemical reactions in mass-action formalism, the original lecture notes of Martin Feinberg

> *M. Feinberg, "Lectures on Chemical Reaction Networks", 1979, available at http://www.chbmeng.ohio-state.edu/∼FEINBERG/LecturesOnReactionNetworks/*

give a complete and rigorous presentation. More recent tutorials written be control theoreticians include

> *David Angeli "A tutorial on chemical reaction network dynamics" Eur. J. Control, 2009, 3-4:398-406*
>
> *Chellaboina, V., Bhat, S. , Haddad, M.M., Bernstein, D.S. "Modeling and analysis of mass-action kinetics". IEEE Control Systems Mag., 29(4):60 - 78, 2009*

The standard reference for Flux Balance Analysis is

> *B. O. Palsson, "Systems Biology", Cambridge Univ. Press, 2006*

Basic gene circuits are probably described in many places, although I did not follow any specific reference. General references for dynamical models in biology, containing much more material, are

> *L. Edelstein-Keshet. "Mathematical Models in Biology", SIAM Classics, 2005*
>
> *E. Sontag, "Lecture Notes in Mathematical Biology", available at the URL: http://www.math.rutgers.edu/∼sontag/613.html*
>
> *B. Ingalls, "Mathematical Modeling in Systems Biology: an Introduction", available at the URL: http://www.math.uwaterloo.ca/∼bingalls/MMSB/*

2 Reaction kinetics

We are interested in dynamical models of complex biochemical reactions. Reactions happen because molecules collide with each other, forming and destroying chemical bonds. If we are interested only in macroscopic effects over a large number of molecules, then we can use the law of mass-action

> **Law of mass-action:** *when 2 or more reactants are involved in a reaction step, the reaction rates are proportional to the product of their concentrations*

The law of mass-action is a semi-empirical law, and find its phenomenological justification as a macroscopic version of collision theory. Constraints to its validity are:

- constant temperature
- compartment in which the reactions happen must be well-mixed
- # of molecules must be high ($\sim 10^{23}$ = n. of Avogadro).

2.1 Models of elementary reactions

The simplest possible reaction one can model is a degradation rate of a molecular species X (meaning: X leaves the compartment of interest, or degrades into products which we are not interested to model). It is represented as

$$X \xrightarrow{k} \emptyset$$

The corresponding ODE is:

$$\frac{dx}{dt} = -kx$$

where x = concentration of X (sometimes written as $x = [X]$), k = rate constant ≥ 0.

Next example is a bimolecular reaction of association: X_3 is the "complex" formed by the binding of X_1 and X_2 (sometimes written as $X_3 = [X_1 X_2]$). The binding happens with a reaction rate constant k

$$X_1 + X_2 \xrightarrow{k} X_3 \tag{1}$$

The mass-action ODEs are:

$$\begin{aligned} \frac{dx_1}{dt} &= -k\, x_1 x_2 \\ \frac{dx_2}{dt} &= -k\, x_1 x_2 \\ \frac{dx_3}{dt} &= k\, x_1 x_2 \end{aligned} \tag{2}$$

The ODEs are nonlinear (multilinear in this case, polynomial in general) and to have a nonambiguous representation one uses a *SR-graph* (Species-Reaction graph), i.e., a bipartite graph with two classes of nodes: molecular species and reactions, see Fig. 1. Notice in (2) that only the molecular species "upstream" of the reaction (i.e., the substrates) enter into the right hand side of the ODE. They enter with a minus sign in the equations for the substrates themselves (their concentration decreases) and with a plus sign for that of the product.

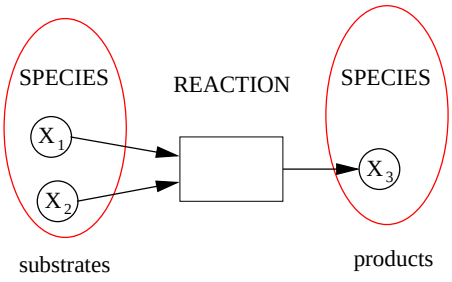


Figure 1: Species-Reactions graph for a single reaction.

The reaction opposite to (2) is a dissociation, and describes the breaking of the complex X_3 into its constituent components:

$$X_3 \xrightarrow{k} X_1 + X_2 \tag{3}$$

The ODEs are :

$$\begin{aligned} \frac{dx_1}{dt} &= k\, x_3 \\ \frac{dx_2}{dt} &= k\, x_3 \\ \frac{dx_3}{dt} &= -k\, x_3 \end{aligned} \tag{4}$$

When both binding/unbinding (2) and (4) can happen simultaneously then we have the reversible association/dissociation

$$X_1 \ + \ X_2 \ \underset{k_2}{\overset{k_1}{\rightleftarrows}} \ X_3 \tag{5}$$

of ODEs:

$$\begin{aligned} \frac{dx_1}{dt} &= -k_1\, x_1 x_2 + k_2\, x_3 \\ \frac{dx_2}{dt} &= -k_1\, x_1 x_2 + k_2\, x_3 \\ \frac{dx_3}{dt} &= k_1\, x_1 x_2 - k_2\, x_3 \end{aligned} \tag{6}$$

When a complex X_3 is formed by several copies of the same substrate (for example p copies of X_1, see Fig. 2) then mass-action law implies that x_1^p enters into the ODEs, and also the rate constant in front of x_1 is modified accordingly. The reaction scheme is

$$pX_1 \ + \ X_2 \ \underset{k_2}{\overset{k_1}{\rightleftarrows}} \ X_3 \tag{7}$$

and the ODEs:

$$\begin{aligned} \frac{dx_1}{dt} &= -p\, k_1\, x_1^p x_2 + p k_2\, x_3 \\ \frac{dx_2}{dt} &= -k_1\, x_1^p x_2 + k_2\, x_3 \\ \frac{dx_3}{dt} &= k_1\, x_1^p x_2 - k_2\, x_3 \end{aligned} \tag{8}$$

p is called a stoichiometric coefficient. Most (but not necessarily all) stoichiometric coefficients are 1. All of them are integers.

The reactions happen in a "compartment" (which for us could be anything from a tank reactor to an *in vivo* organism). The compartment is "open" when there is inflow/outflow of a specie and closed otherwise. A system can be open w.r.t. one specie and closed w.r.t. other species.

Example The "network" of reactions

$$\begin{aligned} X_1 \ + \ X_2 \ &\underset{k_2}{\overset{k_1}{\rightleftarrows}} \ X_3 \\ X_1 \ &\underset{k_4}{\overset{k_3}{\rightleftarrows}} \ \emptyset \end{aligned} \tag{9}$$

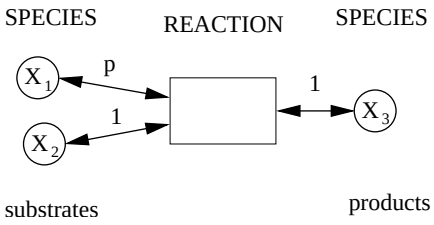


Figure 2: Species-Reactions graph with stoichiometric coefficients.

is similar to (6) and in addition the system is open w.r.t. x_1 (x_1 is produced and degraded), but not w.r.t. x_2 and x_3. The ODEs become (compare with (6)):

$$\begin{aligned}\frac{dx_1}{dt} &= -k_1\, x_1 x_2 + k_2\, x_3 - k_3\, x_1 + k_4 \\ \frac{dx_2}{dt} &= -k_1\, x_1 x_2 + k_2\, x_3 \\ \frac{dx_3}{dt} &= k_1\, x_1 x_2 - k_2\, x_3\end{aligned} \tag{10}$$

Notice in (10) that the inflow is a constant (independent of the concentration of x_1) while the outflow is a first order degradation term.

2.2 Representing biochemical networks through their stoichiometry

Consider a biochemical network involving n molecular species through r reactions. Call

$$x = \begin{bmatrix} x_1 \\ \vdots \\ x_n \end{bmatrix} \in \mathbb{R}^n_+$$

the vector of concentrations of the molecular species. Then $x \in \mathbb{R}^n_+$ because concentrations cannot be negative! Assume that for the r reactions the substrates and products of each reaction (with their stoichiometric coefficients) are known. These data can be though of as the entries of a *stoichiometric matrix*

$$S \in \mathbb{R}^{n \times r}.$$

Each row of S corresponds to a molecular species and each column corresponds to a reaction. If we look at S column-wise, there is a minus sign in front of the stoichiometric coefficients of the substrates (species forming the substrates of a reaction are depleted by the reaction, hence the reaction must contribute a negative term in the ODEs). The products instead have a plus sign. Row-wise, instead, the nonzero entries of the i-th row of S correspond to the reactions in which x_i is involved either as substrate (with a $-$ sign) or as a product (with a $+$ sign). For example for (7)-(8)

$$S = \begin{bmatrix} -p & p \\ -1 & 1 \\ 1 & -1 \end{bmatrix}$$

while for (9)-(10):

$$S = \begin{bmatrix} -1 & 1 & -1 & 1 \\ -1 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 \end{bmatrix}. \tag{11}$$

Reversible reactions means linearly dependent columns in S (equal up to the sign).

To each reaction we can associate a *flux* $v_i(x,k)$, i.e., a compact expression of the kinetics action (for us mass-action) involving the species "upstream" of the reaction (and therefore which enter into the corresponding term of the ODE). Denote

$$v(x,k) = \begin{bmatrix} v_1(x,k) \\ \vdots \\ v_r(x,k) \end{bmatrix}$$

the vector of such reaction fluxes. k is a vector of parameters, representing the reaction rate constants. There is normally one rate constant per reaction, and these are nonnegative:

$$k = \begin{bmatrix} k_1 \\ \vdots \\ k_r \end{bmatrix} \in \mathbb{R}^r_+.$$

Then the ODEs for the biochemical network can be compactly expressed as

$$\frac{dx}{dt} = S\, v(x,k) \tag{12}$$

i.e., as a system of polynomial ODEs. For example, for (7)-(8), $v_1(x,k) = k_1\, x_1^p x_2$ and $v_2(x,k) = k_2\, x_3$ so that (8) can be written as

$$\frac{dx}{dt} = \begin{bmatrix} -p & p \\ -1 & 1 \\ 1 & -1 \end{bmatrix} \begin{bmatrix} k_1\, x_1^p x_2 \\ k_2\, x_3 \end{bmatrix}$$

Notice that in (12) S contains the whole information about the topology of the network. Under the mass-action assumption, an arbitrarily complex network of biochemical reactions can be expressed in this way once S is given.

If in S reversible reactions are all broken down into irreversible forward and backward reactions as e.g. in (11) then $v(x,k) \geq 0$.

To unveil completely the structure of the system of ODEs (12), it is convenient to introduce a further vector $z(x)$ of "complexes", intending with that all compounds of species appearing upstream and downstream of an arrow in a reaction diagram, represented as mass-action terms. For example, in (7) the complexes are $pX_1 + X_2$ and X_3 and

$$z(x) = \begin{bmatrix} x_1^p x_2 \\ x_3 \end{bmatrix}.$$

In other words, $z(x)$ contains all basic multinomial terms appearing in $v(x,k)$, plus the "zero complex" (represented by a 1) to capture the inflow-outflow from the compartment. Assume m is

the dimension of $z(x)$, $m \leq 2r$. Each reaction flux of $v(x,k)$ is obtained multiplying one of the multinomials by the corresponding reaction rate in k: $v_i(x,k) = k_i z_j(x)$. To select from the vector $z(x)$ the term $z_j(x)$ entering into $v_i(x,k)$, we need an "index matrix" $\mathcal{I} : \mathbb{R}^m \to \mathbb{R}^r$ to map the complexes $z(x)$ into the fluxes $v(x,k)$. The entire system of ODEs is then given by the composite map

species		complexes		indexed compexes		fluxes		ODEs
$\mathbb{R}^n$	$\to$	$\mathbb{R}^m$	$\to$	$\mathbb{R}^r$	$\to$	$\mathbb{R}^r$	$\to$	$\mathbb{R}^n$
x	$\mapsto$	$z(x)$	$\mapsto$	$\mathcal{I}\, z(x)$	$\mapsto$	$v(x,k) = \mathrm{diag}(k)\, \mathcal{I}\, z(x)$	$\mapsto$	$\dot{x} = S\, \mathrm{diag}(k)\, \mathcal{I}\, z(x)$

The only nonlinear step is the first, all others are linear maps.

Example Let us look at the example (9)-(10). In this case $n = 3$ and $r = 4$. The complexes are the following 4 compounds appearing in the reaction diagram (9): $\{X_1 + X_2,\, X_1,\, X_3,\, \emptyset\}$. Written in mass-action form, then,

$$z(x) = \begin{bmatrix} x_1 x_2 \\ x_1 \\ x_3 \\ 1 \end{bmatrix}$$

are all multinomial terms in the ODEs (10). If we list the 4 reactions according to the indices of the rate constants k_i given in (9), then the stoichiometric matrix is given in (11), and the index matrix is

$$\mathcal{I} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

Computing explicitly $\dot{x} = S\, \mathrm{diag}(k)\, \mathcal{I}\, z(x)$ leads to (10). In particular the stoichiometric map is a linear map $S : \mathbb{R}^r \to \mathbb{R}^n$ and contains all the information on the topology of the system and on its dynamics.

2.3 Dynamical properties

Consider the biochemical reaction network

$$\frac{dx}{dt} = S\, v(x,k) = S\, \mathrm{diag}(k)\, \mathcal{I}\, z(x) \tag{13}$$

Notice in the composition map above that all parameters of the models (i.e., k) are concentrated in a single step of the cascade. We want to study the dynamical behavior of the system *independently from the numerical value of these parameters*. In the composition map above it can be observed that all parameters are concentrated in the map $\mathrm{diag}(k) : \mathbb{R}^r \to \mathbb{R}^r$. Since $k > 0$, this map is always a bijection, hence from a "structural" point of view it is irrelevant (trivial).

2.3.1 Invariance in $\mathbb{R}^n_+$

The vector x represents concentrations of molecular species and as such it must be and remain nonnegative.

Theorem 2.1 *If $x(0) \in \mathbb{R}^n_+$ then the solution of (13) is such that $x(t) \in \mathbb{R}^n_+ \; \forall\, t \geq 0$ and $\forall\, k \geq 0$.*

To "prove" this theorem, it is enough to observe that all negative terms in the ODEs for x_i vanish when x_i crosses the zero axis (i.e., the negative terms of the ODEs are homogeneous in x) hence $\dot{x}_i$ can never become negative if $x(0) \geq 0$. The positive terms are not required to be homogeneous in x_i. For example the inflow terms by definition are positive constants.

2.3.2 Conservation laws and left kernel of S

Consider the example (6). The ODEs are clearly redundant. If we sum the first and third, or first and second equations

$$\begin{array}{lll} \dfrac{d(x_1+x_3)}{dt} = 0 & \Longrightarrow & x_1(t) + x_3(t) = \text{const} \quad \forall\, t \geq 0 \\ \dfrac{d(x_2+x_3)}{dt} = 0 & \Longrightarrow & x_2(t) + x_3(t) = \text{const} \quad \forall\, t \geq 0 \end{array} \tag{14}$$

which implies that $x_1(t) + x_3(t)$ and $x_2(t) + x_3(t)$ are constants of motion of the dynamical system (6). These constants express conservation of the total amount of a specie: x_1, by itself or bound with x_2 (in the form of the complex x_3), is conserved throughout the evolution, and similarly for x_2. Calling ξ_1 and ξ_2 the two constants in (14), by writing $x_1 = \xi_1 - x_3$ and $x_2 = \xi_2 - x_3$, the system (6) can be reduced to

$$\begin{aligned} \frac{dx_3}{dt} &= k_1\,(\xi_1 - x_3)(\xi_2 - x_3) - k_2\, x_3 \\ x_1 &= \xi_1 - x_3 \\ x_2 &= \xi_2 - x_3 \end{aligned}$$

i.e., each conservation law allows to replace an ODE with an algebraic equation (to be solved off-line). Notice that assigning the initial condition x_o to the system ξ_1 and ξ_2 are uniquely identified. Changing the initial conditions also the ξ_i change.

Let us look at conservation laws for the general formulation (12). the left null space of S, $\ker(S^T) = \{c \in \mathbb{R}^n \text{ s. t. } S^T c = 0\}$ is a vector subspace representing all conservation laws of the biochemical network. Assume $\text{rank}(S) = q \leq \min(n, r)$. Then $\dim(\ker(S^T)) = n - q$, i.e., the system (12) has $n - q$ constants of motion. If $c_1, \ldots, c_{n-q}$ are vectors forming a basis of $\ker(S^T)$, then $N_\ell = \begin{bmatrix} c_1 & \ldots & c_{n-q} \end{bmatrix}^T$ is such that $N_\ell S = 0$. But then $N_\ell \dot{x} = 0$ and therefore, integrating, $N_\ell x(t) = \text{const} = \xi \in \mathbb{R}^{n-q}$ is a systematic expression of the constants of motion of the system. This can be used to reduce the dimension of (12) to q ODEs and $n - q$ algebraic equations of $x(t)$. In fact, if $N_\ell = \begin{bmatrix} N_{\ell,1} & N_{\ell,2} \end{bmatrix}$ with $(N_{\ell,2}) \in \mathbb{R}^{n-q,n-q}$ invertible, from the block splitting $\begin{bmatrix} N_{\ell,1} & N_{\ell,2} \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix} = \xi$ one has $x_2 = N_{\ell,2}^{-1}(\xi - N_{\ell,1} x_1)$, and the ODEs for x_2 can be dropped. Alternatively, one can say that the presence of conservation laws foliates $\mathbb{R}^n$ into *stoichiometric*

classes invariant for the dynamics. Each stoichiometric class is uniquely identified by the initial condition x_o and is expressed as the affine space

$$\mathcal{SC}(x_o) = \{x_o + \mathrm{Im}(S)\} \cap \mathbb{R}^n_+$$

Given x_o, the evolution of (12) is necessarily living in $\mathcal{SC}(x_o)$ for all times: $x(t) \in \mathcal{SC}(x_o)\ \forall\, t \geq 0$.

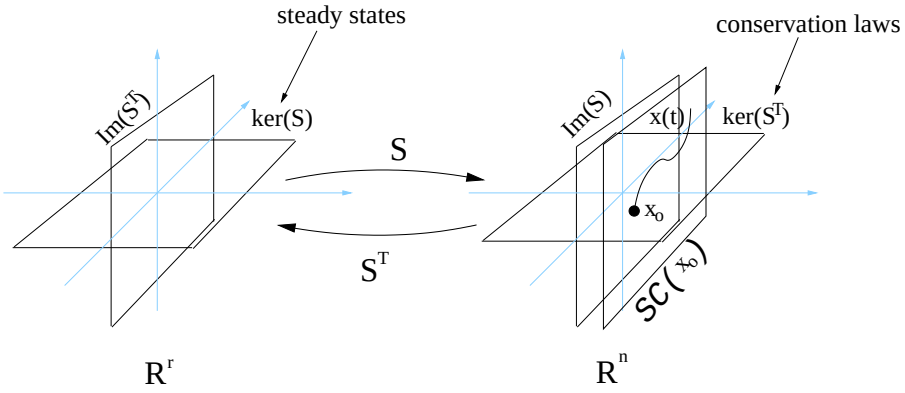


Figure 3: Stoichiometric map, with its subspaces and translated subspaces.

2.3.3 Steady states and right kernel of S

In terms of the fluxes $v(x,k) \in \mathbb{R}^r_+$, the steady states of the system lies in the vector space $\ker(S) = \{v \in \mathbb{R}^r \text{ s. t. } Sv = 0\}$. From $\mathrm{rank}(S) = q$, $\dim(\ker(S)) = r - q$. Since we have broken reversible reactions into pairs of irreversible reactions, we have imposed $v(x,k) \geq 0$ and our S has "twin" columns (differing only in sign, as in (11)) in correspondence of each pair of arrows "$\rightleftarrows$". If all reactions are reversible, it is not complicated to show that this representation can be translated instead into an S with half columns and $v(x,k)$ that can assume any sign (i.e., the reversibility is "swapped" from the stoichiometry to the fluxes). If not all reactions are reversible, however, $\ker(S)$ should be restricted accordingly as $\ker(S) \cap \mathbb{R}^r$, leading in general to a convex cone in the space of fluxes. More details on this in Section 3.

2.3.4 Equilibria and stability

We are interested in investigating the existence of (positive) equilibria, counting them, and understanding their stability properties. In particular, we want to study these properties independently of the numerical values of the parameters k. To analyze these properties, we need to introduce another class of graphs associated to the reaction network (13): the *C-graph* (Complex graph), whose nodes are the complexes and whose edges are the reactions. We must assume that the C-graphs are in "normal form" i.e., each complex label appears only once. Compare (a) and (b) of Fig. 4.

A reaction network is said weakly reversible $\exists$ a directed path of reactions connecting any two nodes of the C-graph (i.e., if the C-graph is strongly connected). For example the C-graph of Fig. 4 (b) is strongly connected, while that of (16) is not, hence the reaction network is not weakly reversible. Call ℓ (= linkage classes) the number of connected components of the C-graph.

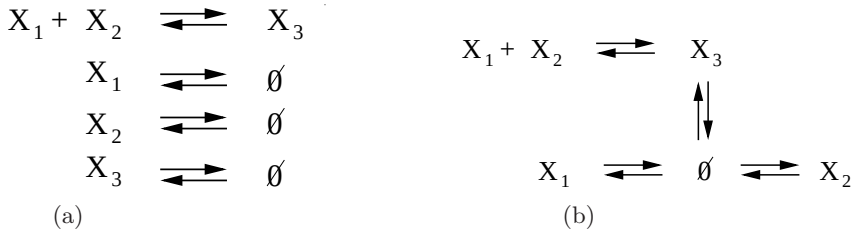


Figure 4: C graph. (a): not in normal form; (b): in normal form.

If $q = \text{rank}(S)$, then $q = \dim(\text{Im}(S))$, i.e., the dimension of the so-called stoichiometric space $\text{Im}(S)$.

We will need an integer index, associated to the structure of a reaction network, called the *deficiency index* δ:

$$\delta := m - \ell - q$$

(recall that m = number of complexes).

Examples

- In the example of Fig. 4

$$S = \begin{bmatrix} -1 & 1 & -1 & 1 & 0 & 0 & 0 & 0 \\ -1 & 1 & 0 & 0 & -1 & 1 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & -1 & 1 \end{bmatrix}$$

  and hence $q = 3$, $\ell = 1$ and $m = 5$, hence $\delta = m - \ell - q = 1$.

- In the example of (16)

$$S = \begin{bmatrix} -1 & 1 & 0 \\ -1 & 1 & 1 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{bmatrix} \tag{15}$$

  i.e., $q = 2$, $\ell = 2$ while $m = 3$. Hence $\delta = m - \ell - q = 0$.

- In the example of (9)-(10), instead, $m = 4$, $\ell = 2$ and $q = 2$, hence $\delta = m - \ell - q = 0$.

The case $\delta = 0$ (*zero deficiency*) is special, because very sharp stability results are available for it. For zero deficiency networks the following theorem in fact holds.

Theorem 2.2 *For any reaction network of zero deficiency we have:*

1. *if the network is* not *weakly reversible then for arbitrary kinetics (i.e., mass-action, Michaelis-Menten, etc.) the system cannot have an equilibrium point in* $\text{int}(R^n_+)$ *and cannot have sustained oscillations.*

2. *if the network is weakly reversible, then for mass-action kinetics the network has a single positive equilibrium point x^* in each stoichiometric class $\mathcal{SC}(x_o)$ and x^* is locally (but conjectured to be "globally") asymptotically stable in $\mathcal{SC}(x_o)$.*

Meaning of 1.: lack of weak reversibility implies that one or more species will disappear asymptotically, hence x^* cannot be positive (i.e., $x^* \notin \text{int}(\mathbb{R}^n_+)$), but it must touch one or more of the axes of $\mathbb{R}^n_+$ (remember that by construction, $x(t)$ nonnegative $\forall\, t \geq 0$).

Meaning of 2.: in each leaf $\mathcal{SC}(x_o)$ in which $\mathbb{R}^n_+$ is foliated, the system has a single equilibrium point in $\text{int}(\mathbb{R}^n_+) \cap \mathcal{SC}(x_o)$ and within $\mathcal{SC}(x_o)$ this equilibrium point is globally asymptotically stable. Notice that since there is a continuum of stoichiometric classes (as we change x_o) there is also a continuum of equilibria, hence, as soon as conservation laws are present, we loose the usual notion of asymptotic stability, because every neighborhood of x^* contains infinitely many other equilibrium points. This notion is sometimes called "semistability", for example in the paper by Chellaboina et. al. mentioned at the begin. (Question: do you see any similarity with the "consensus" problem nowadays very popular??). Only when there are no conservation laws we have the "usual" asymptotic stability concept ($\mathbb{R}^n_+$ lies all in one stoichiometric class in this case). A simple way to avoid conservation laws is to have inflow/outflow for all reactions.

Example: enzyme-catalyzed reaction Most reactions need to be catalyzed by an enzyme to take place at interesting rates. Enzymes are proteins that convert specific substrates into products while remaining basically unchanged. Consider the single substrate - single product reaction shown

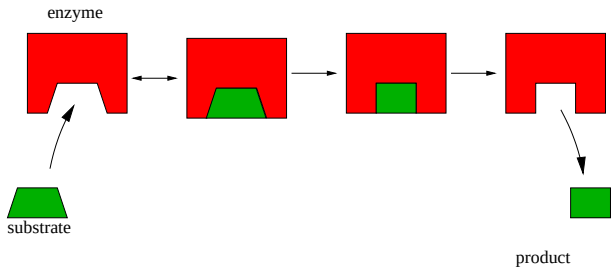


Figure 5: Sketch of an enzyme-catalyzed reaction

in Fig. 5, whose reaction diagram is

$$X_1 + X_2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} X_3 \xrightarrow{k_3} X_2 + X_4 \tag{16}$$

The meaning of the molecular species in this case is:

- X_1 = substrate
- X_2 = enzyme
- X_3 = complex "substrate + enzyme" (" $= [X_1\, X_2]$")
- X_4 = product

Overall the process describes the transformation of the substrate X_1 into the product X_4. The first step is binding/unbinding of the substrate to the enzyme, and it is followed by the catalytic step

which is irreversible. The ODEs are

$$\begin{aligned}\frac{dx_1}{dt} &= -k_1 x_1 x_2 + k_2 x_3\\ \frac{dx_2}{dt} &= -k_1 x_1 x_2 + (k_2 + k_3) x_3\\ \frac{dx_3}{dt} &= k_1 x_1 x_2 - (k_2 + k_3) x_3\\ \frac{dx_4}{dt} &= k_3 x_3\end{aligned}$$

Let us compute the equilibria explicitly. From $\dot{x}_4 = 0 \Longrightarrow x_3 = 0$. Consequently, from $\dot{x}_1 = 0 \Longrightarrow x_1 x_2 = 0$. Hence $x^* \notin \text{int}(\mathbb{R}^4_+)$. The meaning is the following: since the substrate x_1 is transformed into product x_4 and not resupplied, $x_1 \to 0$ and consequently also $x_2 \to 0$ as $t \to \infty$. Indeed the reaction network is not weakly reversible and hence, from Theorem 2.2, it does not admit a positive equilibrium. Notice that there are two conservation laws in the system: $x_2 + x_3 = \xi_1$ and $x_1 + x_3 + x_4 = \xi_2$.

Example (9)-(10) The network is reversible, $\delta = 0$, hence Theorem 2.2 applies and predicts that, in each stoichiometric class, the system has a unique positive equilibrium point which is asymptotically stable for all points in $\mathcal{SC}(x_o)$. Let us compute explicitly the equilibrium/a. From (10)

$$\frac{dx_3}{dt} = 0 \quad \Longrightarrow \quad x_3 = \frac{k_1}{k_2} x_1 x_2$$

Plugging into $\frac{dx_1}{dt} = 0$, we get $x_1 = \frac{k_4}{k_3}$, hence

$$x_3 = \frac{k_1 k_4}{k_2 k_3} x_2 \tag{17}$$

Eq. (17) apparently says that there is an entire ray of equilibria in the (x_2, x_3) plane, see Fig. 6. However, $q = \text{rank}(S) = 2 \Longrightarrow n - q = 3 - 2 = 1 \Longrightarrow \exists$ a conservation law. $\ker(S^T)$ is generated for example by

$$c = \begin{bmatrix} 0 \\ 1 \\ 1 \end{bmatrix}$$

meaning that the constant of motion is determined by $x_2 + x_3 = \xi$ or

$$x_3 = \xi - x_2 \tag{18}$$

In the plane (x_2, x_3) this constant of motion intersects (17) in a single point, see Fig. 6, meaning that on $\mathcal{SC}(x_o)$ the system has indeed a unique equilibrium point. Changing x_o means changing the value of the constant ξ, hence "sliding" the constraint (18) (i.e., passing to another stoichiometric class).

For networks of higher deficiency ($\delta > 0$) other conditions exist, although they are mostly focused on studying the "capacity for multistationarity" i.e., the possibility that for some choice of the parameters k the system may exhibit multiple equilibria in $\mathbb{R}^n_+$. As they are usually formulated as necessary but not sufficient conditions for multistationarity, they are not directly constructive, although algorithms exist to test them.

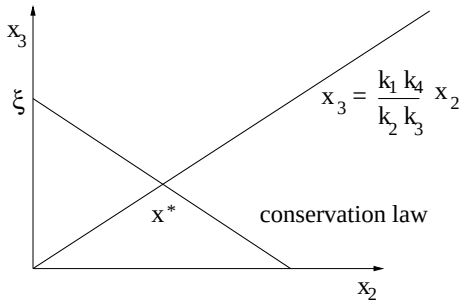


Figure 6: Steady states and conservation laws: 2D-slice of the phase plane for example (9)-(10)

3 Flux Balance Analysis

In the context of metabolic networks (i.e., of networks of biochemical reactions constituting the metabolism of an organism), the idea of flux balance analysis is to disregard the dependence from x (and k) in $v(x,k)$. In this way $\frac{dx}{dt} = Sv$ is not really a system of ODEs (x no longer appears on the r.h.s.), but one can still concentrate on the properties of the stoichiometric map

$$\begin{aligned} S : \mathbb{R}^r_+ &\to \mathbb{R}^n \\ v &\mapsto \frac{dx}{dt} \end{aligned}$$

and in particular study the steady state flux distributions. The *rationale* behind the choice of steady states is that the time constants of the metabolic reactions are very short ($\sim 10^{-1}$ sec) when compared to most other time constants of an organism (for example transcriptional processes have time constants $\sim 10^2 - 10^4$ sec, and protein synthesis/degradation even longer), hence we can assume that the concentration of the metabolites equilibrates fast, i.e. $\frac{dx}{dt} = 0$. We can therefore limit ourselves to study the configurations of fluxes compatible with this assumption. $Sv = 0$ implies $v \in \ker(S)$.

3.1 The cone of steady state fluxes

The fact that $v \geq 0$ implies that steady state fluxes must in reality obey to the set of constraints:

$$\begin{aligned} Sv &= 0 \\ v &\geq 0 \end{aligned} \tag{19}$$

Combining the two constraints, we have $v \in \ker(S) \cap \mathbb{R}^r_+$, that is, the steady state fluxes must belong to a *polyhedral convex cone*. A polyhedral convex cone in $\mathbb{R}^{r-q}_+$ is described as a nonnegative combination

$$\mathcal{C} = \{v \in \mathbb{R}^{r-q} \text{ s. t. } v = \sum_{i=1}^{p} \alpha_i w_i, \quad \alpha_i \geq 0\}$$

where w_i, $i = 1, \ldots, p$, are the generating vectors (or extreme rays). Even if $\dim(\ker(S)) = r - q$ with $q = \text{rank}(S)$, the cone $\mathcal{C}$ is often described by a number of generating vectors p much larger than $r - q$. The extreme rays are called extreme pathways, as they represent pathways on the

reaction graph of the network. Their calculation is a hard computational problem: for networks in which $n, r \sim 10^3$ the number of extreme pathways can be $p \sim 10^6$ or higher.

Example Consider the network of Fig. 7. The stoichiometric matrix is

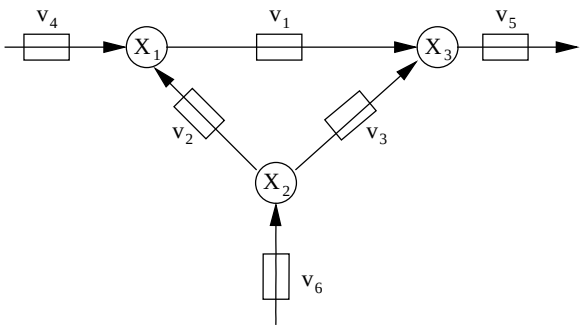


Figure 7: A basic reaction network

$$S = \begin{bmatrix} -1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & -1 & 0 \\ 0 & -1 & -1 & 0 & 0 & 1 \end{bmatrix}$$

has $\text{rank}(S) = 3 \Longrightarrow \dim(\ker(S)) = 6 - 3 = 3$. Consider the 3 vectors $w_i \in \ker(S)$

$$w_1 = \begin{bmatrix} 1 \\ 1 \\ -1 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \qquad w_2 = \begin{bmatrix} 0 \\ 0 \\ 1 \\ 0 \\ 1 \\ 1 \end{bmatrix}, \qquad w_3 = \begin{bmatrix} 1 \\ 0 \\ 0 \\ 1 \\ 1 \\ 0 \end{bmatrix}$$

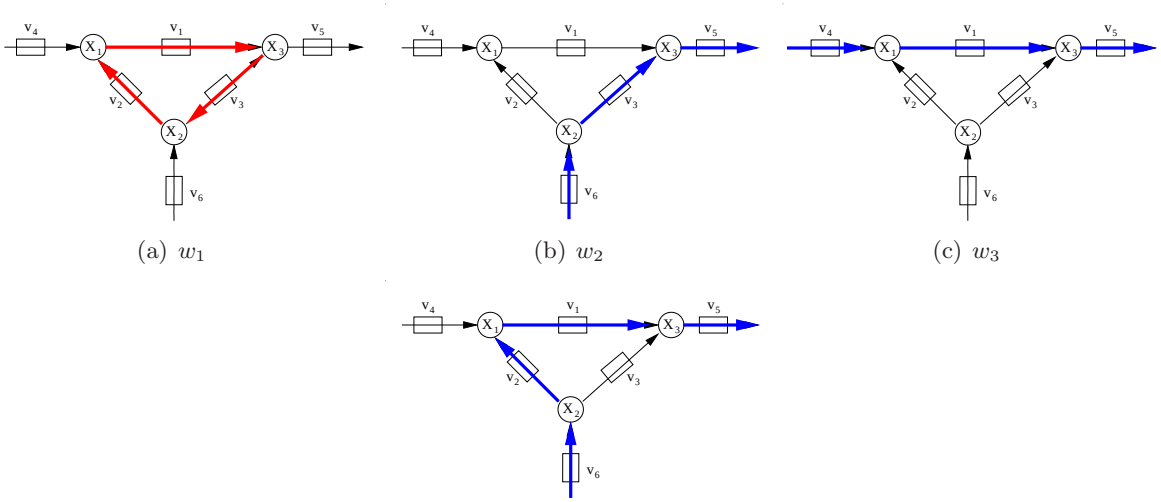
Clearly $\text{span}(w_1, w_2, w_3) = \ker(S)$; however the 3 vectors are not all extreme rays of the cone $\mathcal{C}$. In fact if we look at the corresponding extreme pathways, shown in Fig. 8 (a), (b), (c), then it can be observed that w_1 is not feasible (look at the direction of the arrows), while w_2 and w_3 are.

In place of w_1 one can use instead

$$w_4 = \begin{bmatrix} 1 \\ 1 \\ 0 \\ 0 \\ 1 \\ 1 \end{bmatrix}$$

for which $\text{span}(w_4, w_2, w_3) = \ker(S)$ but also

$$\mathcal{C} = \left\{ v = \begin{bmatrix} w_4 & w_2 & w_3 \end{bmatrix} \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_3 \end{bmatrix}, \quad \alpha_i \geq 0 \right\}.$$



(a) w_1 (b) w_2 (c) w_3

(d) w_4

Figure 8: Nonadmissible (red) and admissible (blue) extremal pathways.

In this case the cone $\mathcal{C}$ is simplicial (i. e. its generators are linearly independent in $\ker(S)$), meaning $p = r - q$. Every steady state flux is then expressed as

$$w_4 = \begin{bmatrix} \alpha_1 + \alpha_3 \\ \alpha_1 \\ \alpha_2 \\ \alpha_3 \\ \alpha_1 + \alpha_2 + \alpha_3 \\ \alpha_1 + \alpha_2 \end{bmatrix}, \qquad \alpha_i \geq 0$$

□

The convex cone $\mathcal{C}$ can be typically restricted to a convex bounded polyhedral set $\mathcal{P}$, by adding further constraints like upper bounds u on the fluxes:

$$\mathcal{P} = \mathcal{C} \cap \{0 \leq v \leq u\}$$

3.2 Choosing a "preferred" steady state flux distribution

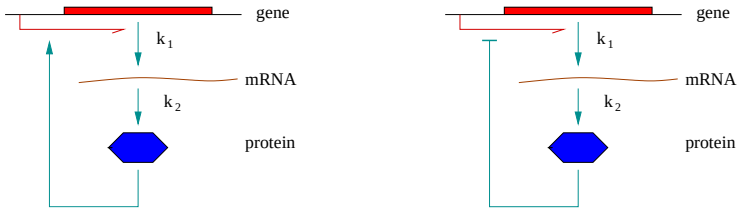
Any $v \in \mathcal{P}$ is an admissible flux distribution. How do we choose a "preferred" flux distribution within $\mathcal{P}$? In "Flux Balance Analysis", one popular choice is to optimize some cost function, for example growth rate i.e., the production of biomass of an organism (the idea is that organisms like bacteria have evolved over millions of years to optimize their growth). This is typically written as a linear cost functional $g(v) = \sum_{i=1}^{r} \beta_i v_i$, where β_i describe the (empirical) relative weights of all reactions which are crucial for growth, such as biosynthesis of nucleotides, aminoacids, fatty acids,

cell-wall components, etc. The problem becomes therefore a Linear Programming problem:

$$\begin{aligned} \max & \sum_{i=1}^{r} \beta_i v_i \\ \text{subject to} \quad & Sv = 0 \\ & 0 \leq v \leq u \end{aligned} \tag{20}$$

4 Gene circuits

Consider the simplest possible gene circuit: an autoregulatory feedback loop in which a gene synthesizes for a protein that acts as a transcription factor influencing the rate of transcription of the gene itself, see Fig. 9.



(a) positive autoregulation (b) negative autoregulation

Figure 9: Autoregulatory gene circuit.

Let us call x_1 = concentration of the gene (i.e., of its mRNA) and x_2 = concentration of the corresponding protein. The transcription factor x_2 binds to a particular region of the DNA, upstream of the region that codes for the gene x_1. This upstream region is called a promoter region. The presence or less of transcription factors attached to this region influences the rate at which the mRNA corresponding to the gene is copied by the cell (i.e., the rate of production of x_1). The influence can be of activator type (in that case we consider the feedback as positive, see Fig. 9 (a)) or of inhibitor type (in this case we consider the feedback as negative, see Fig. 9 (b)).

Let us consider the following basic ODEs to describe this process of autoregulation:

$$\begin{aligned} \frac{dx_1}{dt} &= k_1\,\phi(x_2) - \delta_1\,x_1 \\ \frac{dx_2}{dt} &= k_2\,x_1 - \delta_2\,x_2 \end{aligned} \tag{21}$$

where

- $k_1,\ k_2$ = production rates constants (of gene and protein respectively)
- $\delta_1,\ \delta_2$ = degradation rates constants.

Clearly $x_1, x_2 \geq 0$ because they represent concentrations. The functional $\phi(x_2)$ expresses the action of the transcription factor x_2 on the production of x_1. For low concentrations of x_2 it is reasonable to assume that this action is linear (low x_2 means most binding sites in the promoter region are empty, hence doubling the free x_2 the effect is roughly double; biologists call this a "first order kinetics"). However, when the concentration of x_2 is high, it is likely that most binding sites on the promoter region are already occupied, hence linearity no longer holds and the term representing the production of x_1 saturates. To represent this saturation behavior it is customary to consider functional forms called Michaelis-Menten curves or Hill curves.

4.1 Michaelis-Menten and Hill functional forms

A Michaelis-Menten functional is given by

$$\phi(x) = \frac{x}{\theta + x} \tag{22}$$

and is shown in Fig. 10. The parameter θ is called the "half-saturation" value. For low x indeed the behavior of $\phi(x)$ is nearly linear ("first order kinetics") while for $x \gg \theta$ the behavior is nearly constant ("zero-order kinetics"), i.e. the response saturates for large x. As $\frac{d\phi(x)}{dx} > 0$ the form of

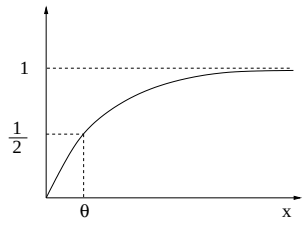


Figure 10: Michaelis-Menten functional.

$\phi(x)$ represents an *activatory* mechanism, although a saturated one. If instead we want to have a saturated *inhibitory* mechanism then we can consider

$$\phi^-(x) = 1 - \phi(x) = \frac{\theta}{\theta + x} \tag{23}$$

This functional is shown in Fig. 11. It saturates at 0 for large x. The slope is negative, hence its inhibitory role. Both (10) and (11) have constant convexity and are called also hyperbolic

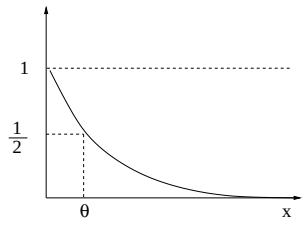


Figure 11: Michaelis-Menten functional, inhibitor version.

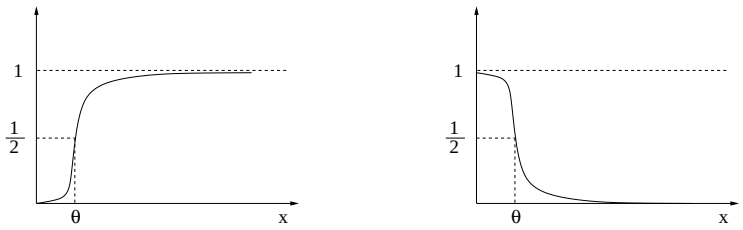
functionals. Sometimes instead it is useful to have functionals whose diagrams exhibit both a convex and a concave part (i.e., sigmoidal curves). In this case the curves commonly used are called Hill curves and are given by the following functionals

$$\phi(x) = \frac{x^h}{\theta^h + x^h}, \qquad h > 1, \quad h \in \mathbb{N} \tag{24}$$

for activatory and

$$\phi^-(x) = 1 - \phi(x) = \frac{\theta^h}{\theta^h + x^h}, \qquad h > 1, \quad h \in \mathbb{N} \tag{25}$$

for inhibitory. The exponent h is called the Hill coefficient. The corresponding curves are shown in Fig. 12. Hyperbolic and sigmoidal curves are compared in Fig 13. In Fig. 14 instead it is shown how a higher h corresponds to a sharper sigmoidal shape. Hill curves are typically associated to (saturated) cooperativity effects, with h representing the "stoichiometry" (i.e., the number of identical molecules entering into a reaction).



(a) positive Hill (b) negative Hill

Figure 12: Hill curves.

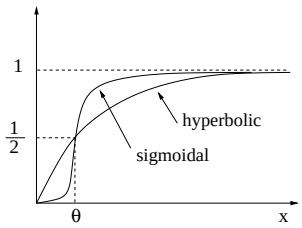


Figure 13: Comparison of hyperbolic and sigmoidal curves.

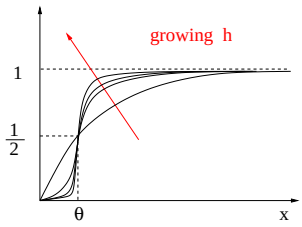


Figure 14: Hill curves for growing exponent h.

4.2 Invariance of $\mathbb{R}^2_+$

The system (21) with any of (22), (23), (24) or (25) is invariant in $\mathbb{R}^2_+$. To see it, it is enough to observe that the "off-diagonal" terms of the ODEs (21) are nonnegative. In particular, $\phi(x) \geq 0$, $\forall\ x \geq 0$ (this is one of the reasons why these functionals are used in the first place, because they guarantee $x(t) \geq 0\ \forall\ t$). The only negative terms in the ODEs are on the diagonal, and vanish when $x_i \to 0$. With an abuse of terminology, one could call the nonlinear system (21) "essentially nonnegative" extending a terminology used for the linear case.

4.3 Positive autoregulation and bistability

Let us consider the system (21) with one of the positive functionals for example the Michaelis-Menten kinetics (22):

$$\begin{aligned} \frac{dx_1}{dt} &= \frac{x_2}{\theta + x_2} - \delta_1 x_1 \\ \frac{dx_2}{dt} &= x_1 - \delta_2 x_2 \end{aligned} \tag{26}$$

where for the sake of simplicity $k_1 = k_2 = 1$.

We now proceed as follows:

- compute the equilibria;
- compute the stability of the equilibria;
- reconstruct the phase portrait of the system.

The system (26) has at most two equilibria:

$$x_0^* = \begin{bmatrix} 0 \\ 0 \end{bmatrix}, \qquad x_1^* = \begin{bmatrix} \frac{1-\delta_1\delta_2\theta}{\delta_1} \\ \frac{1-\delta_1\delta_2\theta}{\delta_1\delta_2} \end{bmatrix}$$

x_0^* corresponds to the situation in which both gene and protein disappear. In order to be biologically consistent, x_1^* must be ≥ 0. That happens when

$$\delta_1\delta_2\theta \leq 1, \tag{27}$$

condition which we assume to hold here. To investigate the stability properties, let us look at the Jacobian linearization of the system (26). The formal Jacobian

$$A = \frac{\partial f}{\partial x} = \begin{bmatrix} -\delta_1 & \frac{\theta}{(\theta+x_2)^2} \\ 1 & -\delta_2 \end{bmatrix}$$

computed at x_0^* yields

$$A_0 = \left.\frac{\partial f}{\partial x}\right|_{x_0^*} = \begin{bmatrix} -\delta_1 & \frac{1}{\theta} \\ 1 & -\delta_2 \end{bmatrix}$$

In $\mathbb{R}^2$, the eigenvalues of a matrix A are given by the formula ($\mathrm{tr}(\cdot) = $ trace)

$$\lambda_{12} = \frac{\mathrm{tr}(A)}{2} \pm \sqrt{\frac{\mathrm{tr}^2(A) - 4\det(A)}{4}}$$

For A_0

$$\begin{aligned} \mathrm{tr}(A_0) &= -(\delta_1 + \delta_2) < 0 \\ \det(A_0) &= \frac{\delta_1\delta_2\theta - 1}{\theta} < 0 \end{aligned}$$

meaning that x_0^* is unstable (it actually is a *saddle point*: eigenvalues are real, one positive the other negative). Notice that as soon as x_1^* becomes non-biologically consistent, i.e., when (27) is violated, then x_0^* becomes asymptotically stable.

Computing the Jacobian matrix at x_1^* (do the calculations...):

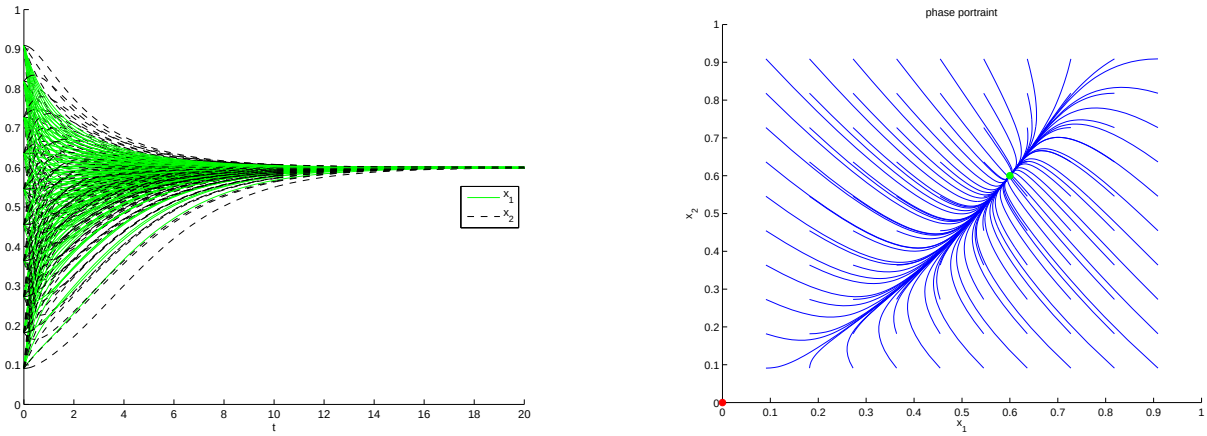
$$A_1 = \left.\frac{\partial f}{\partial x}\right|_{x_1^*} = \begin{bmatrix} -\delta_1 & \theta\delta_1^2\delta_2^2 \\ 1 & -\delta_2 \end{bmatrix}$$

In this case

$$\begin{aligned} \mathrm{tr}(A_1) &= -(\delta_1 + \delta_2) < 0 \\ \det(A_1) &= \delta_1\delta_2(1 - \theta\delta_1\delta_2) > 0 \end{aligned}$$

which imply that x_1^* is asymptotically stable whenever it is admissible.

The trajectories and phase portrait of the system (26) are shown in Fig. 15. All trajectories in $\mathbb{R}^2_+$ tend towards x_1^* (shown in green in Fig. 15 (b)), while x_0^* (red dot) is unstable for all trajectories of $\mathbb{R}^2_+$. In fact, its stable submanifold is outside $\mathbb{R}^2_+$, hence uninteresting for us. Its unstable submanifold is instead along the curve connecting x_0^* to x_1^*, hence trajectories starting near x_0^* are attracted towards x_1^*.



(a) time course

(b) phase portrait

Figure 15: Positive autoregulation, Michaelis-Menten kinetics.

If instead of the Michaelis-Menten functional we use an activator Hill functional, for example with Hill coefficient $h = 2$, then the system becomes:

$$\begin{aligned} \frac{dx_1}{dt} &= \frac{x_2^2}{\theta^2 + x_2^2} - \delta_1\, x_1 \\ \frac{dx_2}{dt} &= x_1 - \delta_2\, x_2 \end{aligned} \tag{28}$$

This system has the following 3 equilibria:

$$x_0^* = \begin{bmatrix} 0 \\ 0 \end{bmatrix}, \qquad x_1^* = \begin{bmatrix} \frac{1-\sqrt{1-4\theta^2\delta_1^2\delta_2^2}}{2\delta_1\delta_2} \\ \frac{1-\sqrt{1-4\theta^2\delta_1^2\delta_2^2}}{2\delta_1} \end{bmatrix}, \qquad x_2^* = \begin{bmatrix} \frac{1+\sqrt{1-4\theta^2\delta_1^2\delta_2^2}}{2\delta_1\delta_2} \\ \frac{1+\sqrt{1-4\theta^2\delta_1^2\delta_2^2}}{2\delta_1} \end{bmatrix}$$

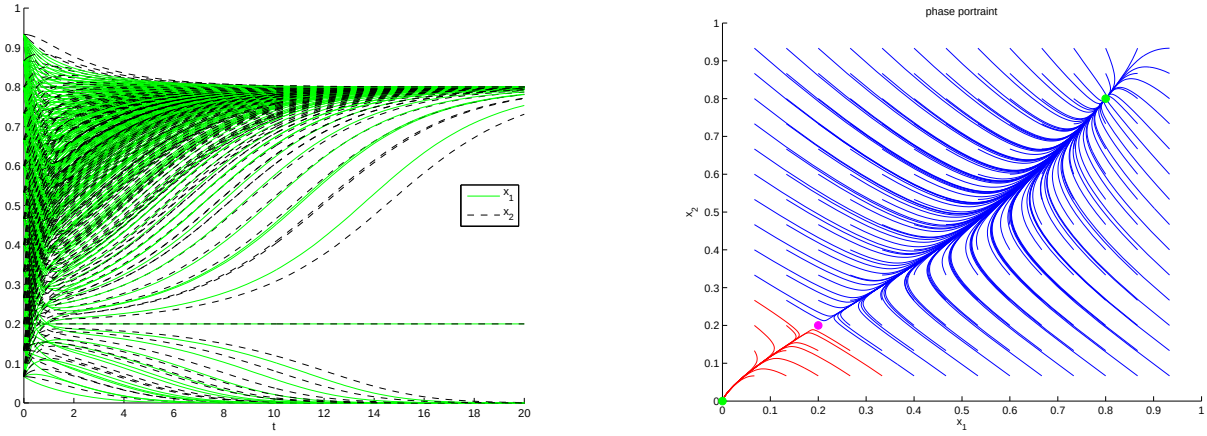
Depending on the values of the parameters, one or 3 of these are biologically admissible equilibria. Assuming $4\theta^2\delta_1^2\delta_2^2 < 1$, we have all 3 in $\mathbb{R}^2_+$. The "formal" Jacobian is now

$$A = \frac{\partial f}{\partial x} = \begin{bmatrix} -\delta_1 & \frac{2x_2\theta^2}{(\theta^2+x_2^2)^2} \\ 1 & -\delta_2 \end{bmatrix}$$

For example, computed in x_0^*

$$A_0 = \left.\frac{\partial f}{\partial x}\right|_{x_0^*} = \begin{bmatrix} -\delta_1 & 0 \\ 1 & -\delta_2 \end{bmatrix}$$

meaning that now x_0^* is asymptotically stable. Doing a similar calculation for the other two equilibria, we obtain that x_1^* is a saddle point and that x_2^* is another asymptotically stable equilibrium. This time, however, the saddle point is strictly inside $\mathbb{R}^2_+$ hence also its stable submanifold (corresponding to the stable eigenvalue) must be in $\mathbb{R}^2_+$. The trajectories and phase portrait of the system (28) are shown in Fig. 16. The trajectories tend towards x_0^* or towards x_2^* (both shown in green in Fig. 16 (b)). The basins of attraction of the two asymptotically stable equilibria are in red and blue. It is clearly visible the existence of a separatrix of the two basins of attraction. This must necessarily correspond to the stable submanifold of the saddle point (the saddle point is shown in magenta in Fig. 16 (b)). The unstable submanifold of the saddle point is also guessable, along the curve that connects x_0^* with x_2^*. The system (28) is a prototype for a bistable system, a widely popular topic in systems biology.



(a) time course

(b) phase portrait

Figure 16: Positive autoregulation, Hill coefficient $h = 2$.

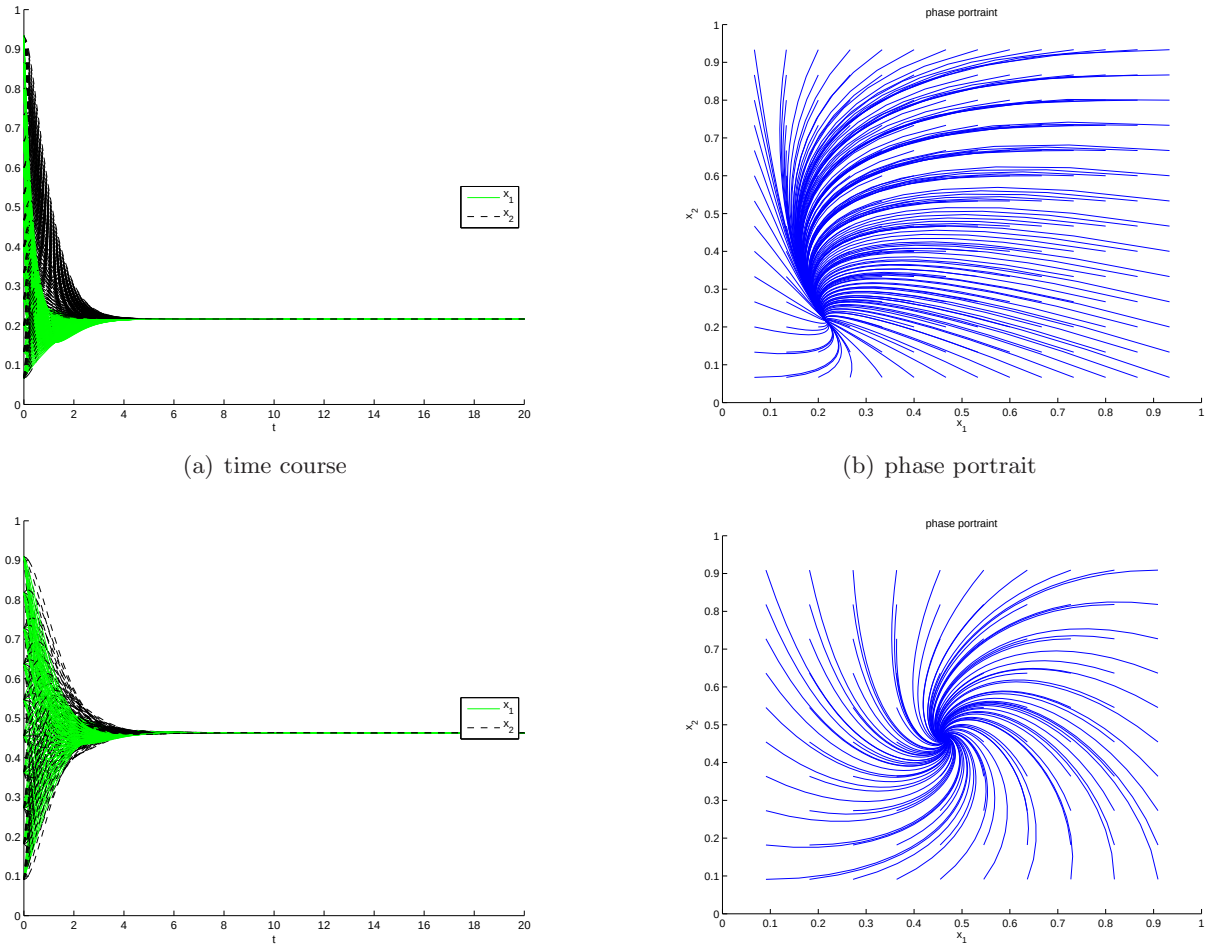
What we deduce from these examples is that postive feedbacks in biology are often not as dangerous as in other domains, because they typically come with saturating effects.

4.4 Negative autoregulation and homeostasis

Let us consider now a case of negative feedback, corresponding for example to the Michaelis-Menten functional (23). The ODES are

$$\begin{aligned} \frac{dx_1}{dt} &= \frac{\theta}{\theta + x_2} - \delta_1 x_1 \\ \frac{dx_2}{dt} &= x_1 - \delta_2 x_2 \end{aligned} \tag{29}$$

The equilibria are given by



(a) time course (b) phase portrait

(c) time course (d) phase portrait

Figure 17: Negative autoregulation, Michaelis-Menten kinetics. In the first row the asymptotically stable equilibrium x_1^* is a sink; in the second row it is a stable spiral.

$$x^* = \begin{bmatrix} \frac{-\theta\delta_1\delta_2 \pm \sqrt{\theta^2\delta_1^2\delta_2^2 + 4\theta\delta_1\delta_2}}{2\delta_1} \\ \frac{-\theta\delta_1\delta_2 \pm \sqrt{\theta^2\delta_1^2\delta_2^2 + 4\theta\delta_1\delta_2}}{2\delta_1\delta_2} \end{bmatrix},$$

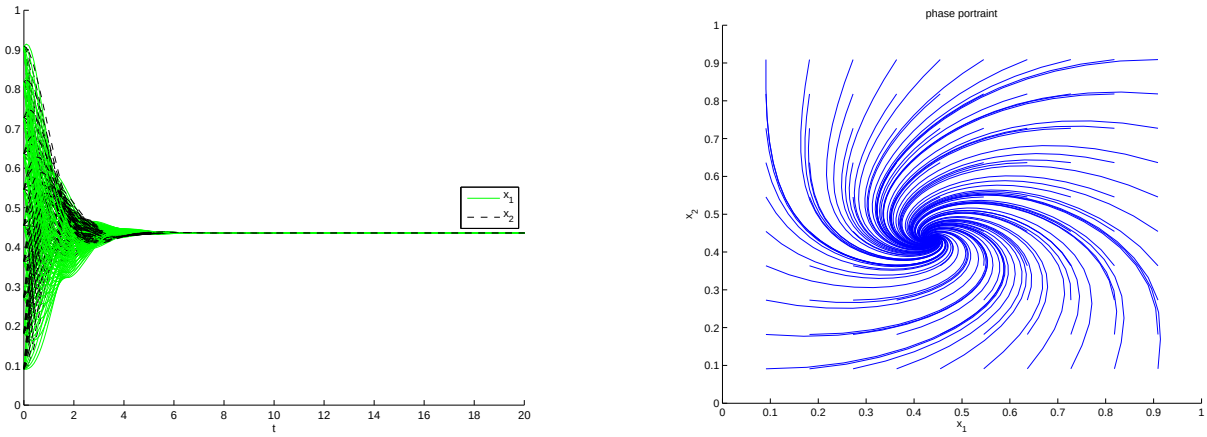
one of which is always positive, call it x_1^*, the other always negative, hence there is always just one biologically admissible equilibrium point. Computing the linearization around this equilibrium, and the eigenvalues of this linearization, then two possibilities emerge, depending on the values of the parameters:

1. the eigenvalues are real negative $\Longrightarrow$ x_1^* is a sink, see first row of Fig. 17;
2. the eigenvalues are complex conjugate with negative real part $\Longrightarrow$ x_1^* is a stable spiral, see second row of Fig. 17.

When we replace Michaelis-Menten with a Hill functional

$$\begin{aligned} \frac{dx_1}{dt} &= \frac{\theta^2}{\theta^2 + x_2^2} - \delta_1 \, x_1 \\ \frac{dx_2}{dt} &= x_1 - \delta_2 \, x_2 \end{aligned} \tag{30}$$

the situation is similar: a single asymptotically stable equilibrium emerges. As an exercise you can try to compute the linearization explicitly and see if the two possibilities mentioned above (sink and stable spiral) are still possible. An example of trajectory/phase portrait is shown in Fig. 18.



(a) time course

(b) phase portrait

Figure 18: Negative autoregulation, Hill coefficient $h = 2$. The asymptotically stable equilibrium is a spiral.

4.5 Other regulatory elements

Many variants of the toy gene circuit shown above are possible. Clearly, the more complex a gene circuit is, the more parameters it can have. Very soon the number of possible (admissible) dynamical features tend to explode. Some basic extra mechanisms are now shown, with the corresponding ODEs. Feel free to study them in detail....

Delayed autoregulation The ODEs for Fig. 19 are

$$\frac{dx_1}{dt} = k_1\,\phi(x_2^\tau) - \delta_1\,x_1$$
$$\frac{dx_2}{dt} = k_2\,x_1^\tau - \delta_2\,x_2$$

where

- $x_1^\tau = x_1(t-\tau_1)$
- $x_2^\tau = x_2(t-\tau_2)$

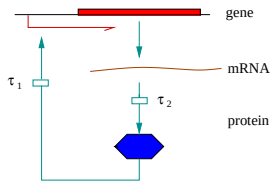


Figure 19: Delayed autoregulation.

Multiple regulation In Fig. 19, two transcription factors act simultaneously, leading to the ODE

$$\frac{dx_g}{dt} = k_g\,\phi_1(x_{pA}) + k_g\,\phi_2^-(x_{pB}) - \delta_g\,x_g$$

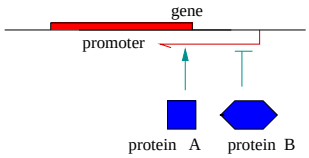


Figure 20: Multiple regulation.

Indirect regulation Fig. 21 shows a case in which transcriptional regulation is mediated by signaling intermediates. Possible ODE are

$$\frac{dx_g}{dt} = k_g\,\phi(x_M) - \delta_g\,x_g$$
$$\frac{dx_E}{dt} = k_E\,x_g - \delta_E\,x_E$$
$$\frac{dx_M}{dt} = k_M\,x_E - \delta_M\,x_M$$

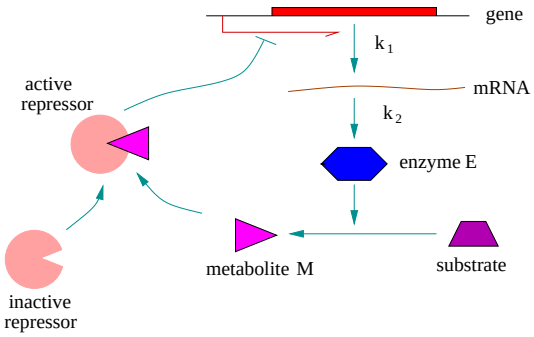


Figure 21: Indirect regulation.