Notes for a Systems Biology course

Reaction Kinetics and Gene Circuits

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October 2015

Contents

These notes are inspired (when not copied) from various sources.
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/

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 by 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

1 Reaction kinetics [Mass-action, Michealis-Menten, Hill]

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).

1.1 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 $X_3 = [X_1X_2]$). 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 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:

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

1.2 Conservation laws

Often in the equations representing reaction kinetics there are conservation laws, representing e.g. conservation of mass. In (6), for instance,

$$\frac{d(x_1 + x_3)}{dt} = 0 \Longrightarrow \quad x_1(t) + x_3(t) = c_1 = \text{const} \quad \forall\, t \geq 0$$
$$\frac{d(x_2 + x_3)}{dt} = 0 \Longrightarrow \quad x_2(t) + x_3(t) = c_2 = \text{const} \quad \forall\, t \geq 0$$

Hence the system (6) reduces to the differential algebraic system

$$\begin{aligned} \frac{dx_3}{dt} &= k_1\,(c_1 - x_3)(c_2 - x_3) - k_2\,x_3 \\ x_1(t) &= c_1 - x_3(t) \\ x_2(t) &= c_2 - x_3(t) \end{aligned}$$

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

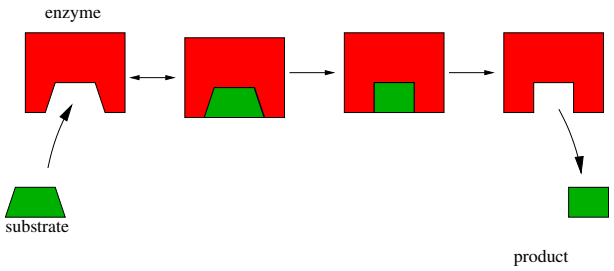


Figure 1: Sketch of an enzyme-catalyzed reaction

in Fig. 1, whose reaction diagram is

$$X_1 + X_2 \underset{k_2}{\overset{k_1}{\rightleftarrows}} X_3 \xrightarrow{k_3} X_2 + X_4 \tag{7}$$

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. According to mass-action, the ODEs are

$$\begin{aligned}
\frac{dx_1}{dt} &= -k_1 x_1 x_2 + k_2 x_3 \\
\frac{dx_2}{dt} &= -k_1 x_1 x_2 + (k_2 + k_3) x_3 \\
\frac{dx_3}{dt} &= k_1 x_1 x_2 - (k_2 + k_3) x_3 \\
\frac{dx_4}{dt} &= k_3 x_3
\end{aligned} \tag{8}$$

or making use of the conservation law (its meaning: the total concentration of enzyme, free or bound, is constant)

$$\frac{d(x_2 + x_3)}{dt} = 0 \quad \Longrightarrow \quad x_2(t) + x_3(t) = c_2 = \text{const} \quad \forall\, t$$

$$\begin{aligned}
\frac{dx_1}{dt} &= -k_1 x_1 (c_2 - x_3) + k_2 x_3 \\
\frac{dx_3}{dt} &= k_1 x_1 (c_2 - x_3) - (k_2 + k_3) x_3 \\
\frac{dx_4}{dt} &= k_3 x_3 \\
x_2 &= c_2 - x_3
\end{aligned} \tag{9}$$

1.3 Quasi steady-state approximation

In the previous example, the concentration of the enzyme X_2 is (much) less abundant than that of the substrate X_1. Hence, after a transient, all molecules of enzyme are used, i.e., substrate is bound to them, and as soon as a reaction is completed the enzyme is re-occupied again by another substrate. In the *quasi steady-state approximation* we make the approximation that the concentration of complex x_3 is constant

$$\frac{dx_3}{dt} = 0$$

from which we obtain in (9)

$$k_1 x_1 (c_2 - x_3) - (k_2 + k_3) x_3 = 0 \quad \Longrightarrow \quad x_3 = \frac{c_2 x_1}{\theta + x_1} \tag{10}$$

where $\theta = \frac{k_1 + k_2}{k_1} > 0$. The functional $\phi(x) = \frac{x}{\theta + x}$ is called a *Michaelis-Menten* function, and it will be analyzed more in detail below. Technically the quasi steady-state approximation corresponds to apply singular perturbation theory to mass-action kinetics.

1.4 Cooperativity

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

$$hX_1 \; + \; X_2 \; \underset{k_2}{\overset{k_1}{\rightleftarrows}} \; X_3 \tag{11}$$

and the mass-action ODEs:

$$\begin{aligned} \frac{dx_1}{dt} &= -p\, k_1\, x_1^h x_2 + hk_2\, x_3 \\ \frac{dx_2}{dt} &= -k_1\, x_1^h x_2 + k_2\, x_3 \\ \frac{dx_3}{dt} &= k_1\, x_1^h x_2 - k_2\, x_3 \end{aligned} \tag{12}$$

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

Example: cooperative enzyme-catalyzed reaction If, as in Fig. 2, the substrate - product

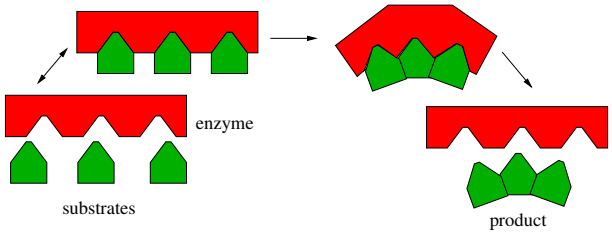


Figure 2: Sketch of a cooperative enzyme-catalyzed reaction, with cooperativity index $p = 4$.

reaction requires h molecules of substrate to be carried out, then we have the reaction diagram

$$hX_1 + X_2 \; \underset{k_2}{\overset{k_1}{\rightleftarrows}} \; X_3 \; \xrightarrow{k_3} \; X_2 + X_4 \tag{13}$$

and the ODEs

$$\begin{aligned} \frac{dx_1}{dt} &= -hk_1 x_1^h x_2 + hk_2 x_3 \\ \frac{dx_2}{dt} &= -k_1 x_1^h x_2 + (k_2 + k_3) x_3 \\ \frac{dx_3}{dt} &= k_1 x_1^h x_2 - (k_2 + k_3) x_3 \\ \frac{dx_4}{dt} &= k_3 x_3 \end{aligned} \tag{14}$$

After the elimination of the conservation laws, the quasi steady-state approximation in this case leads to

$$x_3 = c\frac{x_1^h}{\theta^h + x_1^h} \tag{15}$$

where the functional $\phi(x) = \frac{x^h}{\theta^h + x^h}$ is called a *Hill* function. The exponent p is called the *Hill coefficient* or the *cooperativity index*. More on Hill functions below.

1.5 Other basic mechanisms

Competitive inhibition The mechanism of competitive inhibition corresponds to a second substrate that can bind to the enzyme, thus preventing the primary substrate from binding and inhibiting the formation of the product. See Fig. 3. The reaction diagram is

$$\begin{aligned} X_1 + X_2 &\underset{k_2}{\overset{k_1}{\rightleftarrows}} X_3 \xrightarrow{k_3} X_2 + X_4 \\ X_5 + X_2 &\underset{k_4}{\overset{k_4}{\rightleftarrows}} X_6 \end{aligned} \tag{16}$$

where

- X_5 = competitive inhibitor
- X_6 = alternative complex formed (" $= [X_5\, X_2]$")

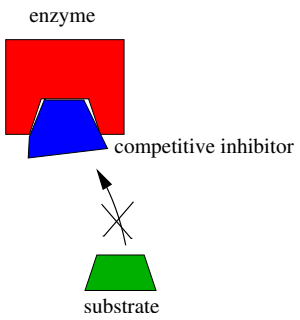


Figure 3: Sketch of an enzyme-catalyzed reaction, with competitive inhibition.

Write down the corresponding ODEs and reduce them using conservation laws and quasi steady-state approximation. What is the difference in terms of product ODE w.r.t. the non-competitive enzyme-catalyzed reaction in (7)?

Example: allosteric inhibition We have an allosteric interaction when the enzyme has a second pocket to which a molecule can bind. If this allosteric binding prevents the primary substrate from binding in its own pocket then we have an allosteric inhibition. See Fig. 4. In this case the reaction

diagram is the same as before

$$\begin{aligned} X_1 + X_2 &\underset{k_2}{\overset{k_1}{\rightleftarrows}} X_3 \xrightarrow{k_3} X_2 + X_4 \\ X_5 + X_2 &\underset{k_4}{\overset{k_4}{\rightleftarrows}} X_6 \end{aligned} \tag{17}$$

Write down the corresponding ODEs and reduce them.

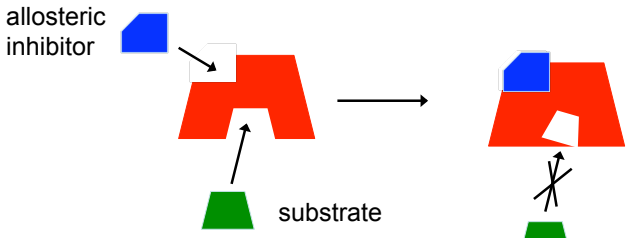


Figure 4: Sketch of an allosterically inhibited enzyme-catalyzed reaction.

1.6 Michaelis-Menten and Hill functional forms

A Michaelis-Menten functional is given by

$$\phi(x) = \frac{x}{\theta + x} \tag{18}$$

and is shown in Fig. 5. 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

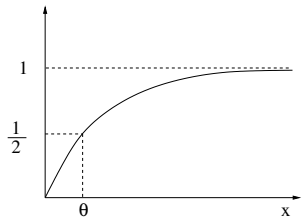


Figure 5: Michaelis-Menten functional.

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

$$\phi^-(x) = 1 - \phi(x) = \frac{\theta}{\theta + x} \tag{19}$$

This functional is shown in Fig. 6. It saturates at 0 for large x. The slope is negative, hence its inhibitory role. Both (5) and (6) have constant convexity and are called also hyperbolic functionals.

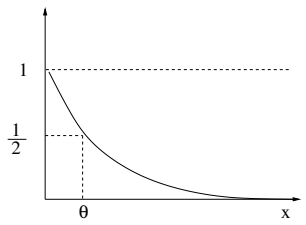


Figure 6: Michaelis-Menten functional, inhibitor version.

Example: enzyme-catalyzed reaction in the quasi steady-state approximation. From (9) and (10) we have that the ODE for the product is

$$\dot{x}_4 = \underbrace{k_3 c_2}_{\text{const}} \frac{x_1}{\theta + x_1}$$

meaning that when the concentration of substrate X_1 is low the reaction rate for the product X_4 is (almost) a linear function of x_1, but it saturates to a max value of $k_3 c_2$ when x_1 is big ($c_2 = x_2 + x_3 =$ total concentration of enzyme molecules).

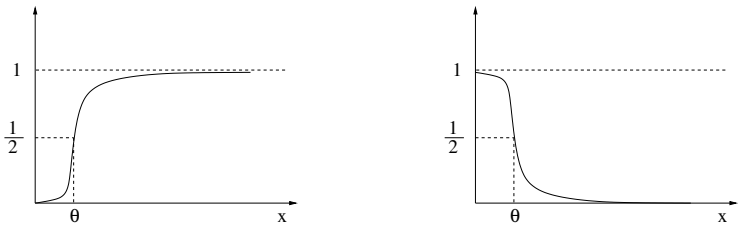
Sometimes instead if Michaelis-Menten curves is useful to have functionals whose diagrams exhibit both a convex and a concave part (i.e., sigmoidal curves). In this case the curves commonly used are the Hill curves given by the following functionals

$$\phi(x) = \frac{x^h}{\theta^h + x^h}, \qquad h > 1, \quad h \in \mathbb{N} \tag{20}$$

for activatory and

$$\phi^-(x) = 1 - \phi(x) = \frac{\theta^h}{\theta^h + x^h}, \qquad h > 1, \quad h \in \mathbb{N} \tag{21}$$

for inhibitory. The exponent h is called the Hill coefficient. The corresponding curves are shown in Fig. 7. Hyperbolic and sigmoidal curves are compared in Fig 8. In Fig. 9 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 7: Hill curves.

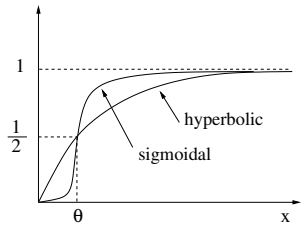


Figure 8: Comparison of hyperbolic and sigmoidal curves.

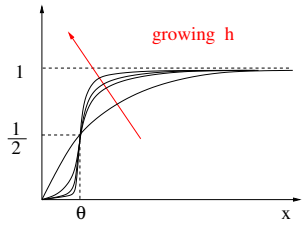


Figure 9: Hill curves for growing exponent h.

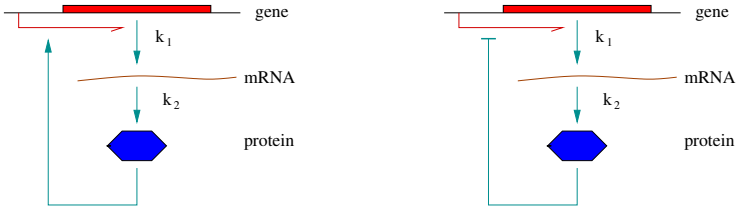
Example: cooperative enzyme-catalyzed reaction in the quasi steady-state approximation. Consider the system (14) with the quasi steady-state approximation (15). The ODE for the product is

$$\dot{x}_4 = \underbrace{k_3 c}_{\text{const}} \frac{x_1^h}{\theta^h + x_1^h}$$

meaning that now at low concentrations x_1 the rate of x_4 is polynomial, not linear. It becomes saturated at high x_1.

2 Gene circuits [Equilibria, Stability and Phase Plane Analysis in 2D]

Consider the simplest possible gene circuit: an autoregulatory feedback loop in which a gene synthesizes a protein that acts as a transcription factor influencing the rate of transcription of the gene itself, see Fig. 10.



(a) positive autoregulation (b) negative autoregulation

Figure 10: 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. 10 (a)) or of inhibitor type (in this case we consider the feedback as negative, see Fig. 10 (b)).

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

$$\begin{aligned} \frac{dx_1}{dt} &= k_1\,\phi(x_2) - \delta_1\,x_1 \\ \frac{dx_2}{dt} &= k_2\,x_1 - \delta_2\,x_2 \end{aligned} \tag{22}$$

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 of Michaelis-Menten or Hill type.

We now proceed as follows:

- show that the ODEs (22) are well-posed (i.e., represent concentrations);

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

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

The system (22) with any of (18), (19), (20) or (21) is invariant in $\mathbb{R}^2_+$. To see it, it is enough to observe that the "off-diagonal" terms of the ODEs (22) 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 (22) "essentially nonnegative" extending a terminology used for the linear case.

2.2 Local stability

2.2.1 Stability of a linear system (recap)

Consider a linear system

$$\dot{x} = Ax \tag{23}$$

and call $\lambda_1, \ldots, \lambda_n$ its eigenvalues. We have that the equilibrium point $x^* = 0$ is

- *asymptotically stable* iff $\mathrm{Re}(\lambda_i) < 0\ \forall\ i$
- *marginally stable* iff $\mathrm{Re}(\lambda_i) \leq 0$ and the eigenvalues λ_i for which $\mathrm{Re}(\lambda_i) = 0$ correspond to Jordan blocks of dimension 1;
- *unstable* otherwise.

2.2.2 Local stability from linearization

For a nonlinear system

$$\dot{x} = f(x) \tag{24}$$

at an equilibrium point x^* such that $f(x^*) = 0$ we can use the Jacobian linearization to investigate local stability. If $A = \frac{\partial f(x^*)}{\partial x}$, of eigenvalues $\lambda_1, \ldots, \lambda_n$, is the Jacobian linearization at x^*, then

- x^* is *locally asymptotically stable* for the nonlinear system (24) if $\mathrm{Re}(\lambda_i) < 0\ \forall\ i$;
- x^* is *unstable* for the nonlinear system (24) if $\mathrm{Re}(\lambda_i) > 0$ for some i;
- if instead $\mathrm{Re}(\lambda_i) \leq 0$ and $\mathrm{Re}(\lambda_i) = 0$ for some i, then the stability character of the nonlinear system is undecidable by looking at the linearization.

2.2.3 A special case: linear phase plane analysis for 2D system

Once the equilibria of (22) have been computed, to investigate their stability properties we can look at the linearization of (22) around an equilibrium. For that we need the Jacobian matrix:

$$A = \frac{\partial f}{\partial x} = \begin{bmatrix} -\delta_1 & k_1 \frac{\partial \phi(x_2)}{\partial x_2} \\ 1 & -\delta_2 \end{bmatrix}.$$

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

$$\lambda_{1,2} = \frac{\mathrm{tr}(A)}{2} \pm \sqrt{\frac{\mathrm{tr}^2(A) - 4\det(A)}{4}} \tag{25}$$

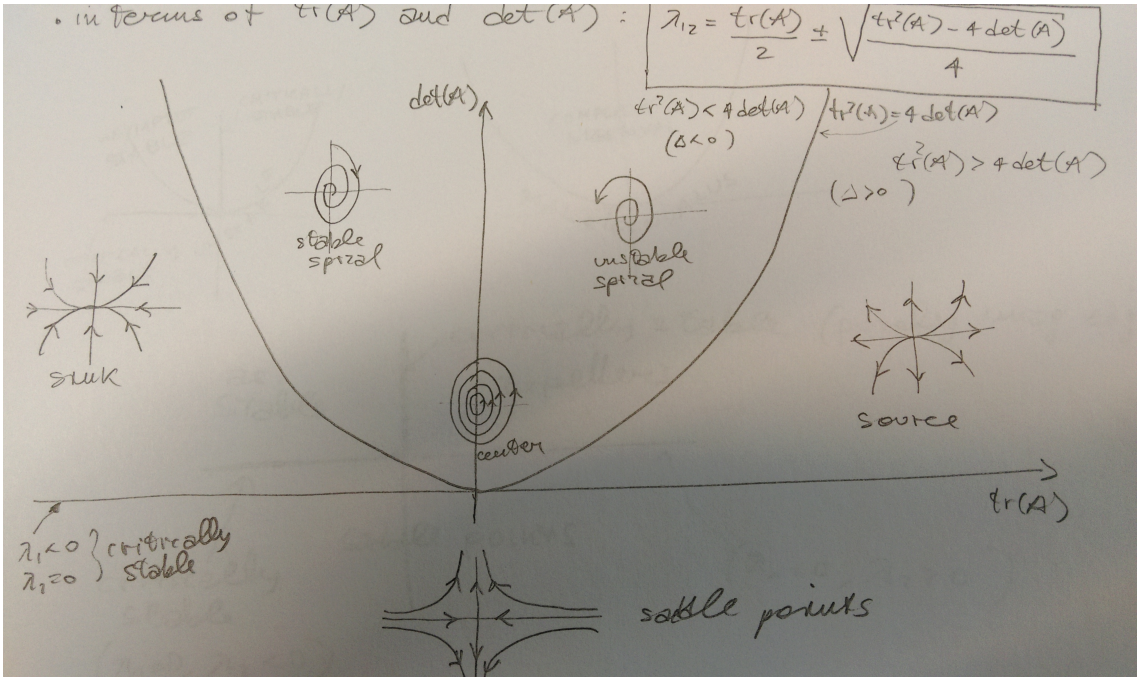


Figure 11: Phase plane analysis in 2D.

The following cases can appear:

- case of $\lambda_{1,2}$ real, non-zero, $\lambda_1 \neq \lambda_2$
  1. $\lambda_1 < 0$, $\lambda_2 < 0 \Longrightarrow$ *sink* (stable node)
  2. $\lambda_1 > 0$, $\lambda_2 > 0 \Longrightarrow$ *source* (unstable node, repeller)
  3. $\lambda_1 < 0$, $\lambda_2 > 0 \Longrightarrow$ *saddle point* (unstable)
- case of $\lambda_1 = 0$ and $\lambda_2 \neq 0$
  1. if $\lambda_2 < 0$ then the linear system is marginally stable (but nothing can be said of the original nonlinear system)

2. if $\lambda_2 > 0$ then the linear system is unstable (but, again, nothing can be said of the original nonlinear system)

- case of $\lambda_{1,2}$ complex conjugate $\lambda_{1,2} = \alpha \pm i\beta$
  1. $\alpha = 0 \Longrightarrow$ *center* (ellipses or circles) $\Longrightarrow$sustained oscillations
  2. $\alpha < 0 \Longrightarrow$ *spiral sink* (stable) $\Longrightarrow$damped oscillations
  3. $\alpha > 0 \Longrightarrow$ *spiral source* (unstable) $\Longrightarrow$increasing oscillations

The values of $\mathrm{tr}(A)$ and $\det(A)$ allows to determine completely the local phase portrait of a linear system having A as its state matrix. The possibilities are shown in Fig. 11. In the $\mathrm{tr}(A) - \det(A)$ plane, stability corresponds to the top-left quadrant, while the other 3 are unstable.

2.3 Positive autoregulation with Michaelis-Menten [Single attractor]

Let us consider the system (22) with the positive functional (18)

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

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

The system (26) has at most two equilibria:

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

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

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

hence using (25) we have that x_0^* is unstable. From the classification of Section 2.2.3 and Fig. 11, we can say that x_0^* is actually 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. Since $\mathrm{tr}^2(A_1) - 4\det(A_1) = (\delta_1-\delta_2)^2 + 4\theta\delta_1^2\delta_2^2 > 0$, from Fig. 11, the equilibrium x_1^* is a sink.

2.3.1 Nullclines and complete phase portrait in 2D

The linearization gives us only a local picture of the flow of the system. In $\mathbb{R}^2$, the analysis of the *nullclines* allows to reconstruct a complete phase portrait of the nonlinear system.

- x_1-nullcline: $\{x \in \mathbb{R}^2_+ \text{ s. t. } \dot{x}_1 = 0\}$, i.e,

$$x_1 = \frac{x_2}{\delta_1(\theta + x_2)}$$

- x_2-nullcline: $\{x \in \mathbb{R}^2_+ \text{ s. t. } \dot{x}_2 = 0\}$, i.e,

$$x_1 = \delta_2 x_2$$

- intersection of the nullclines: equilibria.

Computing the flow of the system on the nullclines:

$$\left.\frac{dx_2}{dt}\right|_{x_1\text{-null}} = \frac{x_2}{\delta_1(\theta+x_2)} - \delta_2 x_2 = \frac{x_2}{\delta_1(\theta+x_2)}(\delta_1\delta_2(x_{1,2}^* - x_2)) \begin{cases} > 0 & \text{if } x_2 < x_{1,2}^* \\ = 0 & \text{if } x_2 = x_{1,2}^* \\ < 0 & \text{if } x_2 > x_{1,2}^* \end{cases}$$

and

$$\left.\frac{dx_1}{dt}\right|_{x_2\text{-null}} = \frac{x_2}{\theta+x_2} - \delta_1\delta_2 x_2 = \frac{x_2}{\theta+x_2}(\delta_1\delta_2(x_{1,2}^* - x_2)) \begin{cases} > 0 & \text{if } x_2 < x_{1,2}^* \\ = 0 & \text{if } x_2 = x_{1,2}^* \\ < 0 & \text{if } x_2 > x_{1,2}^* \end{cases}$$

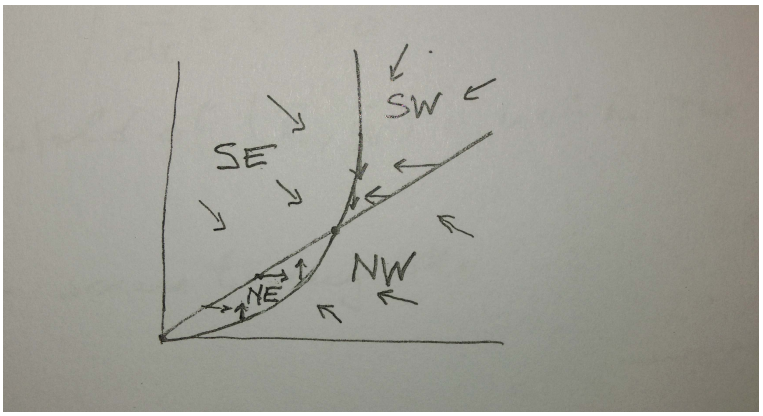


Figure 12: Nonlinear phase plane analysis in 2D for positive autoregulation with Michaelis-Menten kinetics.

The key observation is that the x_1 and x_2 components of the flow of the system can "change direction" only on the nullclines, hence by knowing the sign of the derivatives on the nullclines we know it (qualitatively) everywhere. The resulting picture is shown in Fig. 12. Notice that the regions indicated "NE" and "SW" in Fig. 12 are trapping regions, i.e., invariant for the flow of the system. Since the origin is a saddle point, this implies that its unstable manifold must be inside the "NE" region. Its stable manifold instead is outside the positive orthant hence we do not consider it. The presence of trapping regions excludes also the possibility of an equilibrium point which is a spiral (and in fact x_1^* is a sink).

Such phase portrait can be verified in simulations. The trajectories and phase portrait of the system (26) are shown in Fig. 13. All trajectories in $\mathbb{R}^2_+$ tend towards x_1^* (shown in green in Fig. 13 (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^*. In conclusion, the entire orthant $\mathbb{R}^2_+$ (except x_0^*) is attracted towards x_1^*.

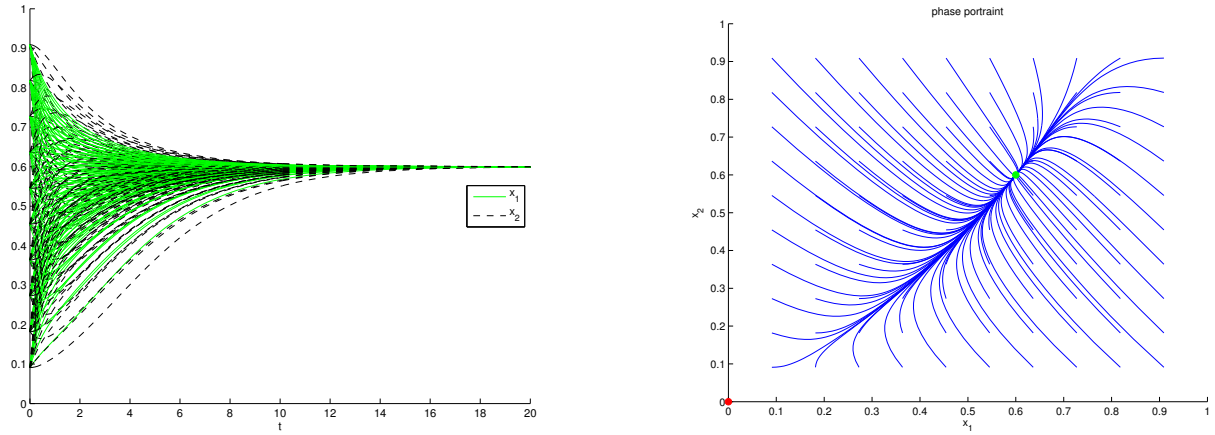
2.4 Positive autoregulation with Hill function [Bistability]

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:

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



(a) time course

(b) phase portrait

Figure 13: Positive autoregulation, Michaelis-Menten kinetics.

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^*

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

and

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

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 $\mathbb{R}^2_+$.

2.4.1 Nullclines and complete phase portrait in 2D

Computing the nullclines for the system (28) we get:

- x_1-nullcline:

$$x_1 = \frac{x_2^2}{\delta_1(\theta^2 + x_2^2)} \tag{29}$$

- x_2-nullcline:

$$x_1 = \delta_2 x_2 \tag{30}$$

- intersection of the nullclines: equilibria.

Computing the flow of the system on the nullclines:

$$\left.\frac{dx_2}{dt}\right|_{x_1\text{-null}} = \frac{x_2^2}{\delta_1(\theta^2+x_2^2)} - \delta_2 x_2 = x_2\frac{x_2 - \delta_1\delta_2(\theta^2+x_2^2)}{\delta_1(\theta^2+x_2^2)} \begin{cases} <0 & \text{if } x_2 \text{ small} \\ >0 & \text{if } x_2 \text{ intermediate} \\ <0 & \text{if } x_2 \text{ big} \end{cases}$$

and similarly for $\left.\frac{dx_1}{dt}\right|_{x_2\text{-null}}$. Hence for the flow in $\mathbb{R}^2_+$ we have the qualitative picture shown in Fig. 14. From the direction of the flow on the nullclines, the 3 regions SW and NE are all trapping regions. Of the 3 equilibrium points, the one in the middle is a saddle point and the other two are locally asymptotically stable. Because of the trapping regions, the unstable manifold of the saddle point has be inside the two trapping regions SW and NE, while the stable manifold of the saddle point must be outside. This stable manifold give rises to a separatrix between the basins of attraction of the two asymptotically stable equilibria: trajectories on the separatrix will stay on the separatrix for all times (and converge to the saddle point), trajectories above the separatrix will converge to x_2^* and trajectories below it will converge to x_0^*. Also in this case, the presence of trapping regions excludes the possibility of eigenvalues with complex conjugate part on the 3 equilibria.

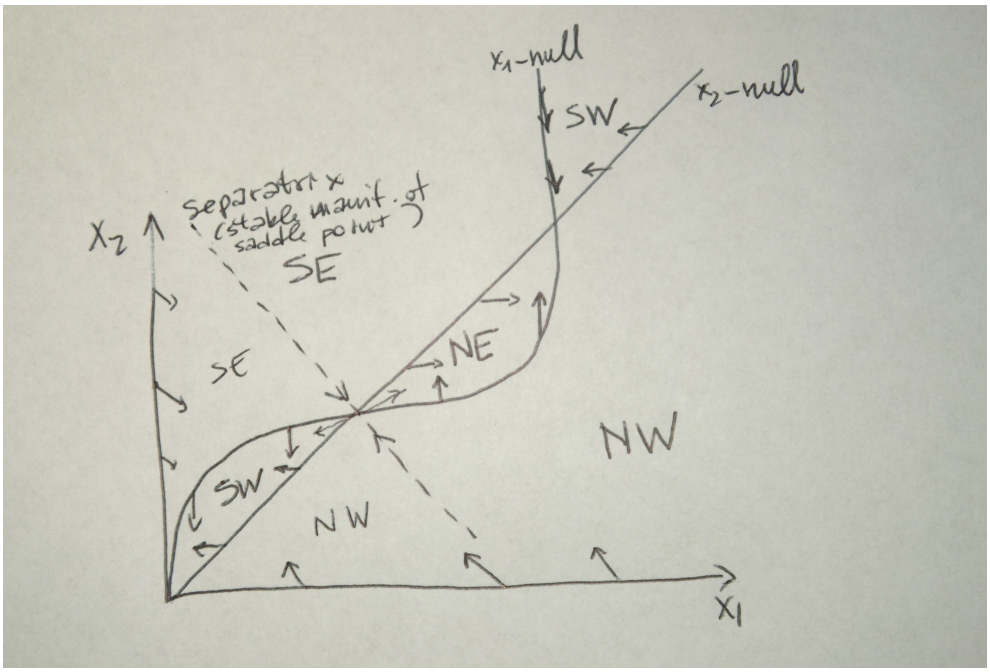
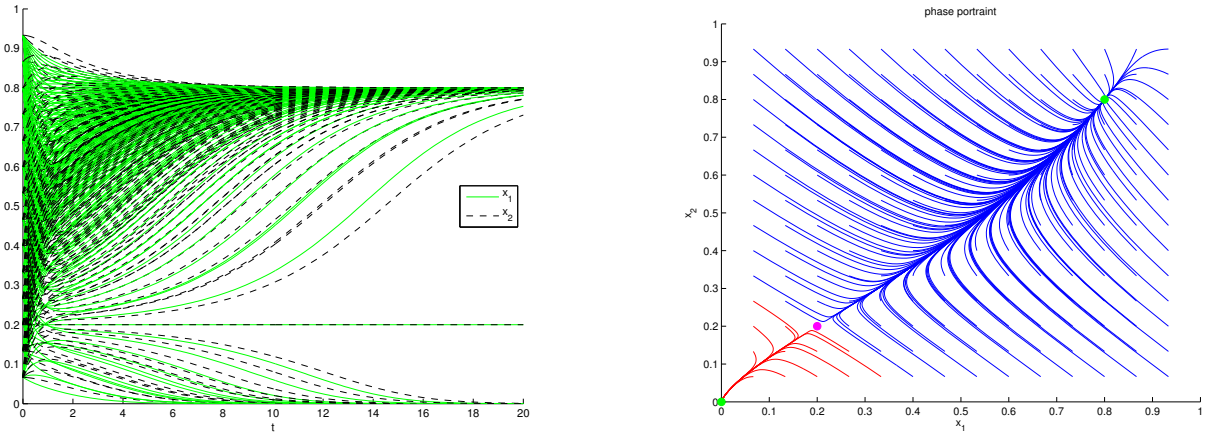


Figure 14: Nonlinear phase plane analysis in 2D for positive autoregulation with Hill kinetics.

Numerical simulations of the trajectories and of the phase portrait of the system (28) are shown in Fig. 15. The trajectories tend towards x_0^* or towards x_2^* (both shown in green in Fig. 15 (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. 15 (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 15: Positive autoregulation, Hill coefficient $h = 2$.

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

2.4.2 Saddle-node bifurcation

Assume we continuously change one of the parameters of the sysem (28), for instance δ_2. As we increase δ_2, the x_2-nullcline (30) changes as shown in Fig. 16, i.e., the number of intersections between the x_1-nullcline and the x_2-nullcline (and hence the number of equilibria of (28)) changes: one observes that the saddle point and one of the stable nodes get close, then collapse into each other and then disappear altogether. This is called a *saddle-node bifurcation*, meaning a qualitative/quantitative change in the number of equilibria of the system. The bifurcation diagram on the right of Fig. 16 shows what happens along the $x_1$coordinate (vertical axis) as we vary the parameter δ_2 (horizontal axis): two of the equilibria (one stable, solid line, and one one unstable, dashed line) approach each other and then disappear.

2.5 Negative autoregulation [Single attractor]

Let us consider now a case of negative feedback, corresponding for example to the Michaelis-Menten functional (19). The ODES are

$$\begin{aligned} \frac{dx_1}{dt} &= \frac{\theta}{\theta + x_2} - \delta_1 x_1 \\ \frac{dx_2}{dt} &= x_1 - \delta_2 x_2 \end{aligned} \tag{31}$$

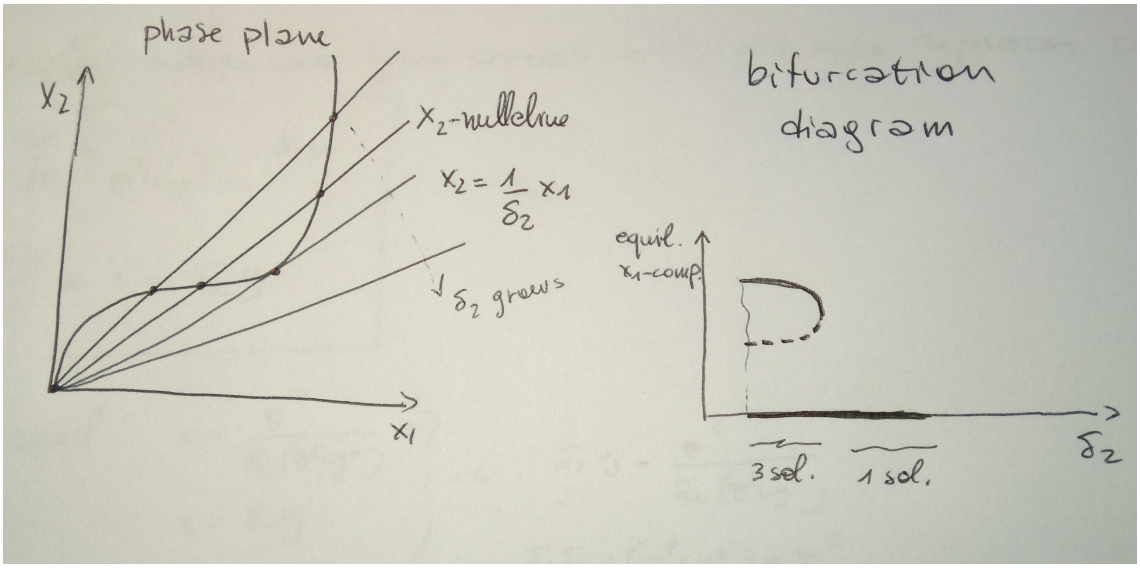


Figure 16: Saddle-node bifurcation for positive autoregulation with Hill kinetics.

The equilibria are given by

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

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. 18;
2. the eigenvalues are complex conjugate with negative real part $\Longrightarrow$ x_1^* is a stable spiral, see second row of Fig. 18.

The nullclines and the phase portrait are shown in Fig. 17. In this case there are no trapping regions around the locally asymptotically stable equilibrium point, which is compatible with the possibility of spiralling trajectories.

When we replace Michaelis-Menten with a Hill functional

$$\begin{aligned} \frac{dx_1}{dt} &= \frac{\theta^2}{\theta^2 + x_2^2} - \delta_1 x_1 \\ \frac{dx_2}{dt} &= x_1 - \delta_2 x_2 \end{aligned} \tag{32}$$

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. 19.

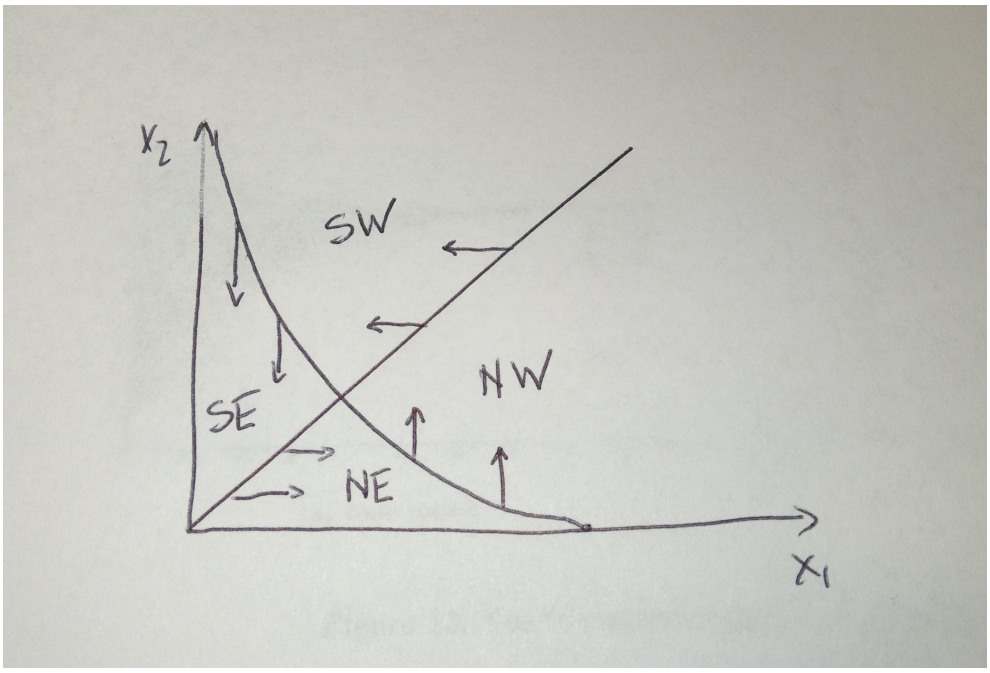


Figure 17: Nonlinear phase plane analysis in 2D for negative autoregulation with Michaelis-Menten kinetics.

2.6 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. 20 are

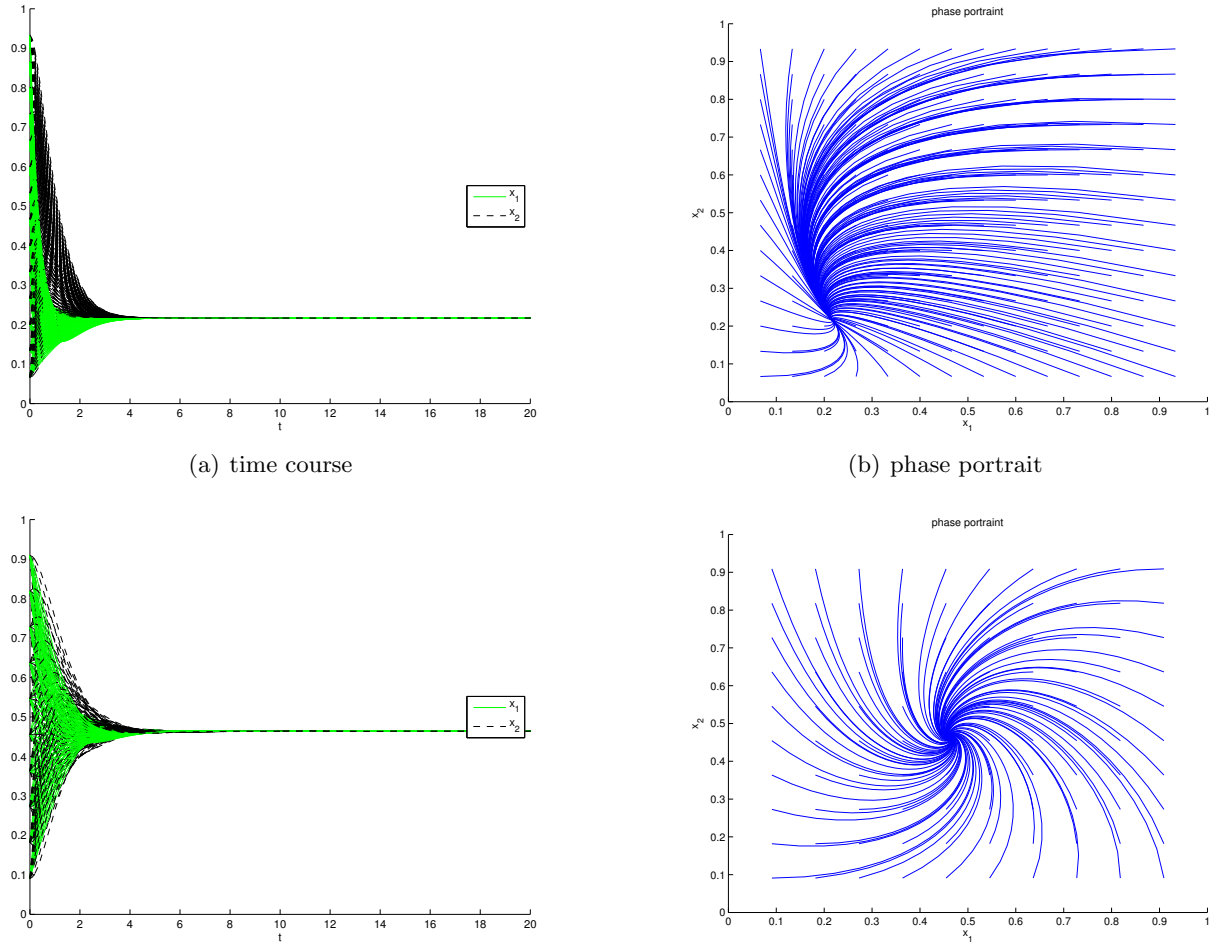
$$\frac{dx_1}{dt} = k_1\,\phi(x_2^\tau) - \delta_1\,x_1$$
$$\frac{dx_2}{dt} = k_2\,x_1^\tau - \delta_2\,x_2$$

where

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

Multiple regulation In Fig. 20, two transcription factors act simultaneously, leading to the ODE

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



(a) time course

(b) phase portrait

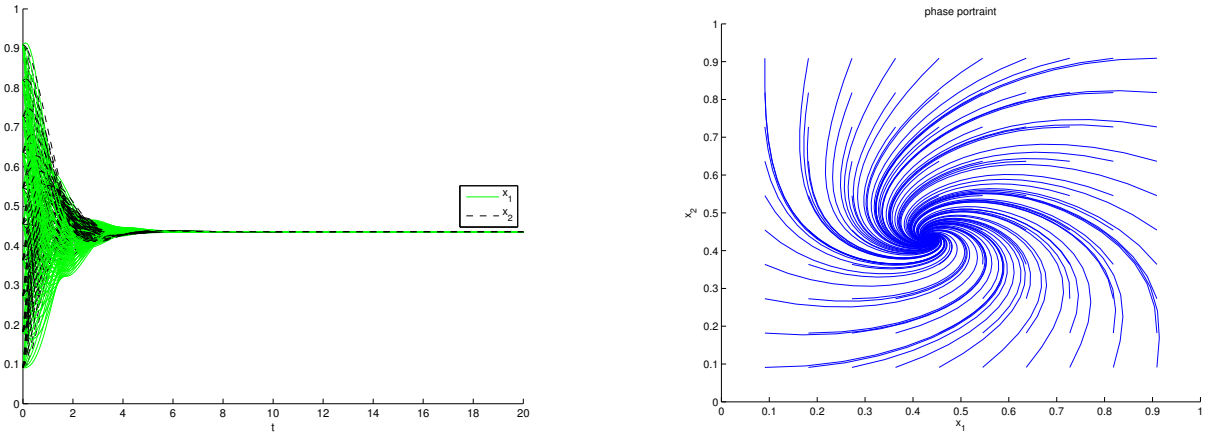
(c) time course

(d) phase portrait

Figure 18: 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.

Indirect regulation Fig. 22 shows a case in which transcriptional regulation is mediated by signaling intermediates. Possible ODE are

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



(a) time course (b) phase portrait

Figure 19: Negative autoregulation, Hill coefficient $h = 2$. The asymptotically stable equilibrium is a spiral.

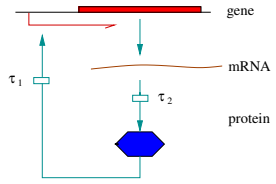


Figure 20: Delayed autoregulation.

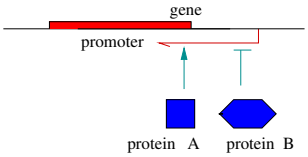


Figure 21: Multiple regulation.

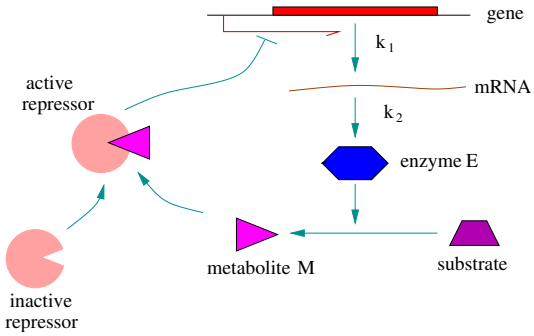


Figure 22: Indirect regulation.