Systems Biology Notes

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1 Palsson. Chap. 1. Introduction

Assumptions

The systems biology approach in this course has a number of assumptions (see Fig. 1).

Table 1.2 Assumptions used in the formulation of biological network models			
Assumption	Description		
(1) Continuum assumption	Do not deal with individual molecules, but treat medium as a continuum		
(2) Finer spatial structure ignored	Medium is homogeneous		
(3) Constant-volume assumption	V is time-invariant, $dV/dt = 0$		
(4) Constant temperature	lsothermal <mark>systems</mark>		
-	Kinetic properties a constant		
(5) Ignore physico-chemical factors	Electroneutrality and osmotic pressure can be important factors, but are ignored		

Figure 1: Assumptions of the systems approach.

System dynamics

The stoichiometric matrix is used to model the reaction topology of a network. Let us denote as x_i as the concentration of the different species. Then, in the following example

$$CP(x_1) \iff PC(x_2)$$

$$C(x_3) + P(x_4) \iff CP(x_1)$$

$$CP(x_1) + A(x_5) \iff AP(x_6) + C(x_3)$$

The differential equation defining the time evolution of the concentrations is given by

$$\frac{d\mathbf{x}}{dt} = S\mathbf{v}(\mathbf{x})$$

where

$$S = \begin{pmatrix} -1 & 1 & -1 \\ 1 & 0 & 0 \\ 0 & -1 & 1 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \\ x_5 \\ x_6 \end{pmatrix}$$

Each column of S represents a reaction and each row is associated to a given compound. We may also check the validity of S through its elemental balance. Each row of the matrix E represents the elemental composition (in our example, P, C, and A) of each one of the compounds involved (sorted by x_i , in our example, $x_1, x_2, ..., x_6$). In our example it would be

$$E = \begin{array}{ccccccccc} x_1 & x_2 & x_3 & x_4 & x_5 & x_6 \\ P & \begin{pmatrix} 1 & 1 & 0 & 1 & 0 & 1 \\ 1 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 \end{pmatrix}$$

If E and S are well constructed, it must be

$$ES = 0$$

Another interesting calculation is the number of non-zero entries by rows and columns

$$S = \begin{pmatrix} 2 & 3 & 4 \\ -1 & 1 & -1 \\ 1 & 0 & 0 \\ 0 & -1 & 1 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} 2 \\ 1 \\ 1 \\ 1 \\ 1 \end{pmatrix}$$

The number of non-zero entries by columns represents the number of different species participating in a reaction. The number of non-zero entries by rows represents the number of reactions in which a particular compound participates (this number is called the connectivity of that compound). Note that S is giving the reaction topology, but not the reaction dynamics. The kinetic properties of the reaction are given by $\mathbf{v}(\mathbf{x})$. For a general chemical reaction

$$dD + mM \leftrightarrow pDM$$

the reaction rate is defined as

$$v = -\frac{1}{d}\frac{dx_D}{dt} = -\frac{1}{m}\frac{dx_M}{dt} = \frac{1}{p}\frac{dx_{DM}}{dt}$$

and it can be calculated in terms of the concentration of the different species as

$$v = k_f x_D^{d'} x_M^{m'} - k_b x_{DM}^{p'}$$

where k_f and k_b are "constants" (they are not really constants because they depend on pressure and temperature) for the forward and backward reactions. For reactions taking place in a single step, the exponents are equal to the stoichiometric coefficients (d' = d, m' = m, p' = p). At equilibrium, the reaction speed is 0 meaning that

$$\begin{array}{rcl}
k_f x_D^{d'} x_M^{m'} - k_b x_{DM}^{p'} &= & 0 \\
k_f x_D^{d'} x_M^{m'} &= & k_b x_{DM}^{p'} \\
K &= & \frac{k_f}{k_b} = \frac{x_{DM}^{p'}}{x_D^{d'} x_M^{m'}}
\end{array}$$

which is the famous equilibrium constant. However, we are not restricted in the differential equation to work at equilibrium conditions.

In our example, the time evolution of the species concentration would be given by

$$\frac{d\mathbf{x}}{dt} = S \begin{pmatrix} k_{f_1}x_1 - k_{b_1}x_2 \\ k_{f_2}x_3x_4 - k_{b_2}x_1 \\ k_{f_3}x_1x_5 - k_{b_3}x_6x_3 \end{pmatrix}$$

However, this differential equation is non-linear which makes its analysis more complicated. It may be linearized through the gradient matrix G

$$G = \frac{d\mathbf{v}(\mathbf{x})}{d\mathbf{x}} = \begin{pmatrix} \frac{\partial v_1}{\partial x_1} & \dots & \frac{\partial v_1}{\partial x_N} \\ \dots & \dots & \dots \\ \frac{\partial v_M}{\partial x_1} & \dots & \frac{\partial v_M}{\partial x_N} \end{pmatrix}$$

In our example

$$G = \begin{pmatrix} k_{f_1} & -k_{b_1} & 0 & 0 & 0 \\ -k_{b_2} & 0 & k_{f_2} & k_{f_2} & 0 & 0 \\ k_{f_3} & 0 & -k_{b_3} & 0 & k_{f_3} & -k_{b_3} \end{pmatrix}$$

With this matrix G, the differential equation is linearized with the Jacobian J = SG

$$\frac{d\mathbf{x}}{dt} = SG\mathbf{x}$$

The right null space of the stoichiometric matrix $(S\mathbf{v} = \mathbf{0})$ provides the reaction speeds needed for a steady-state solution. In our example

$$S\mathbf{v} = \mathbf{0} \Rightarrow \mathbf{v} = \mathbf{0} \Rightarrow \begin{cases} k_{f_1}x_1 = k_{b_1}x_2\\ k_{f_2}x_3x_4 = k_{b_2}x_1\\ k_{f_3}x_1x_5 = k_{b_3}x_6x_3 \end{cases}$$

The left null space of the stoichiometric matrix $(\mathbf{l}^T S = \mathbf{0}^T \Rightarrow S^T \mathbf{l} = \mathbf{0})$ provides time invariants. The reason is that

$$\begin{array}{rcl} \frac{d\mathbf{x}}{dt} &=& S\mathbf{v}\\ \mathbf{l}^T \frac{d\mathbf{x}}{dt} &=& \mathbf{l}^T S \mathbf{v}\\ \frac{d(\mathbf{l}^T \mathbf{x})}{dt} &=& \mathbf{0}^T \mathbf{v}\\ \frac{d(\mathbf{l}^T \mathbf{x})}{dt} &=& 0\\ \mathbf{l}^T \mathbf{x} &=& ct \end{array}$$

In our example,

$$S^{T}\mathbf{l} = \mathbf{0} \Rightarrow \mathbf{l} = (l_{3} - l_{5} + l_{6}, l_{3} - l_{5} + l_{6}, l_{3}, -l_{5} + l_{6}, l_{5}, l_{6})$$

A basis of this subspace is given by

$$\{(1, 1, 1, 0, 0, 0), (-1, -1, 0, -1, 1, 0), (1, 1, 0, 1, 0, 1)\}$$

However, having negative coefficients make the interpretation of the time invariants more complicated. So we look for another basis with non-negative coefficients

 $\{(1, 1, 1, 0, 0, 0), (1, 1, 1, 0, 1, 1), (1, 1, 0, 1, 0, 1)\}$

That is the time invariants of this system are given by

$$x_1 + x_2 + x_3 = ct$$

$$x_1 + x_2 + x_3 + x_5 + x_6 = ct$$

$$x_1 + x_2 + x_4 + x_6 = ct$$

or what is the same

$$[CP] + [PC] + [C] = ct$$

$$[CP] + [PC] + [P] + [A] + [AP] = ct$$

$$[CP] + [PC] + [P] + [AP] = ct$$

The first equation states the fact that the concentration of all compounds containing C is constant (C is taking part of CP, PC or alone). Similarly, the third one states the same for P. The second one states that all the A and P consumed are used to produce CP, PC and AP compounds in the rest of the network.

The equation

$$\begin{array}{rcl} \frac{d\mathbf{x}}{dt} &=& S\mathbf{v}\\ \frac{d\mathbf{x}}{dt} &=& \mathbf{s}_1v_1 + \mathbf{s}_2v_2 + \dots \end{array}$$

implies that $\frac{d\mathbf{x}}{dt}$ is in the column space of S, that is, \mathbf{x} can only move in the directions imposed by the columns of S. If instead of the columns of S, we look at the rows of S we have

$$\begin{pmatrix} \frac{dx_1}{dt} \\ \frac{dx_1}{dt} \\ \dots \end{pmatrix} = \begin{pmatrix} \mathbf{r}_1^T \\ \mathbf{r}_2^T \\ \dots \end{pmatrix} \mathbf{v} = \begin{pmatrix} \mathbf{r}_1^T \mathbf{v} \\ \mathbf{r}_2^T \mathbf{v} \\ \dots \end{pmatrix}$$

Equivalently

$$\frac{dx_i}{dt} = \mathbf{r}_i^T \mathbf{v} = \|\mathbf{r}_i\| \|\mathbf{v}\| \cos \theta$$

This equation means that the concentration of a given compound does not change if the system is at equilibrium $(||\mathbf{v}|| = 0)$ or the velocity vector is "aligned" with the *i*-th row of the stoichiometric matrix. The inner product $\mathbf{r}_i^T \mathbf{v}$ is measuring unbalances in the composition of the *i*-th compound with respect to its equilibrium concentration.

2 Palsson. Chap. 2. Basic concepts

<u>Time constants</u>

Let us consider the differential equation

$$\frac{dx}{dt} = -kx \quad x(0) = x_0$$

Its solution is given by

$$x(t) = x(0)e^{-kt} = x(0)e^{-\frac{t}{\tau}}$$

The constant

$$\tau = \frac{1}{k}$$

is called the time constant and it is the time required for the concentration x(t) to fall to 36.8% (= 1/e) of its initial value. Larger k values are associated to faster processes (τ is smaller). The half-life time constant (time for the concentration to reduce to 1/2) is

$$\tau_{1/2} = \frac{\log(2)}{k}$$

Let us consider the first three reactions of glycolysis:

$$G \xrightarrow{HK} G6P \xleftarrow{PGI} F6P \xrightarrow{PFK} FDP$$

Glucose (G) is converted by an hexokinase (HK) into glucose-6-phosphate (G6P), which is converted into fructose-6-phosphate (F6P) by a phosphoglucoseisomerase (PGI). Then, another kinase (PFK) converts F6P into fructose-1,6biphosphate (FDP). The isomerase is much faster than the two kinases. So, if we are analyzing the system at a long period of time we may consider the $G6P \leftrightarrow F6P$ part of the system to be at equilibrium and pool both species together into a single variable (Hexosephosphate pool, HP = G6P + F6P). At this time scale, the system would look like

$$G \xrightarrow{HK} HP \xrightarrow{PFK} FDP$$

Reaction rate

Consider the reaction

$$S \to P$$

We may have **zero-order reaction** rates, that is the rate of formation of the product is independent of the amount of reactant. Zero-order reactions are typically found when a material that is required for the reaction to proceed, such as a surface or a catalyst, is saturated by the reactants. In that case,

$$v = k$$

Integrating the concentration of substrate and product over time, we have

$$\begin{array}{rcl} x_S(t) &=& x_S(0) - kt \\ x_P(t) &=& kt \end{array}$$

The half-life constant is

$$\tau_{1/2} = \frac{x_S(0)}{2k}$$

We have **first-order reaction** rates when the amount of product depends linearly on the concentration of the reactant. For instance

$$S \rightarrow P_1 + P_2$$

Then

$$v = \frac{dx_{P_1}}{dt} = \frac{dx_{P_2}}{dt} = -\frac{dx_S}{dt} = kx_S$$

$$x_S(t) = x_S(0)e^{-kt}$$

$$x_{P_1}(t) = x_{P_2}(t) = x_S(0)(1 - e^{-kt})$$

The half-life constant is

$$\tau_{1/2} = \frac{\log(2)}{k}$$

Second-order reactions are of the kind $2A \rightarrow P$ or $A + B \rightarrow P$. The reaction rate is in the first case

$$v = \frac{dx_P}{dt} = -\frac{1}{2}\frac{dx_A}{dt} = kx_A^2$$

and

$$v = \frac{dx_P}{dt} = -\frac{dx_A}{dt} = -\frac{dx_B}{dt} = kx_A x_B$$

in the second case. The solution of the first case is

$$\frac{1}{x_A(t)} = \frac{1}{x_A(0)} + kt$$

There is no closed-form solution for x_A and x_B , but there is an interesting relationship between the two (when $x_A(0) \neq x_B(0)$).

$$\log \frac{x_B(t)x_A(0)}{x_B(0)x_A(t)} = k(x_B(0) - x_A(0))t$$

The half-life constants in the first case is

$$\tau_{1/2} = \frac{1}{kx_A(0)}$$

The half-life constants in the second case are different for A and B and cannot be easily defined.

A second-order reaction, $A + B \rightarrow P$ may be considered a **pseudo first-order reaction** if one of the reactants is much more abundant than the other. For instance, let's assume that B is much more abundant than A. Then, the reaction rate

$$v = kx_A x_B \approx (kx_B(0))x_A = k' x_A$$

It behaves as a first-order reaction, with half-life constant $\tau_{1/2} = \frac{\log(2)}{k'} = \frac{\log(2)}{k'}$

 $\overline{kx_B(0)}^{kx_B(0)}$. Enzymatic reactions

Let us consider the enzymatic reaction

$$S + E \xleftarrow{k_f}{k_b} SE \xrightarrow{k_{cat}} P + E$$

We may write the differential equations associated to these reactions

$$\frac{\frac{dx_S}{dt}}{\frac{dx_E}{dt}} = -k_f x_S x_E + k_b x_{SE}$$

$$\frac{\frac{dx_E}{dt}}{\frac{dt}{dt}} = -k_f x_S x_E + k_b x_{SE} + k_{cat} x_{SE}$$

$$\frac{\frac{dx_{SE}}{dt}}{\frac{dt}{dt}} = k_f x_S x_E - k_b x_{SE} - k_{cat} x_{SE}$$

$$(1)$$

The enzyme is not consumed at the reaction, so its concentration must remain constant over time

$$x_E(t) + x_{SE}(t) = x_E(0)$$

Michaelis-Menten assumed that the first reaction was very fast, meaning that it was at equilibrium for all practical purposes. Then,

$$k_f x_S x_E = k_b x_{SE}$$

Using the enzyme conservation law

$$k_f x_S(x_E(0) - x_{SE}) = k_b x_{SE} \Rightarrow x_{SE} = \frac{x_E(0)x_S}{K_d + x_S}$$

where $K_d = \frac{k_b}{k_f}$ is the dissociation constant of the substrate-enzyme complex. The velocity of the reaction, the speed at which the product is formed is given by

$$v = \frac{dx_P}{dt} = k_{cat} \frac{x_E(0)x_S}{K_d + x_S} = \frac{v_{max}x_S}{K_d + x_S}$$

where $v_{max} = k_{cat} x_E(0)$.

Briggs and Haldane assumed that the concentration of the substrateenzyme complex did not change on the time-scale of product formation (this is called, the quasi-steady-state assumption, QSSA), then

$$k_f x_S x_E = k_b x_{SE} + k_{cat} x_{SE}$$

Combining this equation with the conservation law of the enzyme, we get (see Fig. 2)

$$x_{SE} = \frac{x_E(0)x_S}{K_m + x_S}$$

where

$$K_m = \frac{k_b + k_{cat}}{k_f}$$

and the velocity of reaction

$$v = \frac{v_{max}x_S}{K_m + x_S}$$

where $v_{max} = k_{cat} x_E(0)$.



Figure 2: Velocity of reaction by QSSA approximation. Michaelis-Menten approximation is similar but using K_d instead of K_m .

Pool variables and reaction rates

Consider the interconversion between ATP, ADP and AMP (see Fig. 3). We may write the associated equation system as

$$\begin{array}{rcl} \frac{dx_{ATP}}{dt} &=& -(v_1+v_{-5})+(v_2+v_5)\\ \frac{dx_{ADP}}{dt} &=& (v_1+2v_{-5})-(v_2+2v_5)\\ \frac{dx_{AMP}}{dt} &=& (v_3+v_5)-(v_4+v_{-5}) \end{array}$$



Figure 3: ATP, ADP and AMP conversion. v_5 and v_{-5} are fast reactions; v_1 and v_2 are intermediate; and v_3 and v_4 are slow reactions.

If we sum all the three equations we have the variation over time of the adenosine phosphates (mono-, di-, and tri-)

$$\frac{d(x_{ATP} + x_{ADP} + x_{AMP})}{dt} = v_3 - v_4$$

This differential equation is controlled by slow time processes (remind that v_3 and v_4 are slow). Another pool variable of interest is the sum of high-energy phosphates (2ATP + ADP)

$$\frac{d(2x_{ATP} + x_{ADP})}{dt} = -v_1 + v_2$$

This aggregate variable depends on intermediately fast reactions. Finally, a fast changing variable can be constructed as

$$\frac{d(x_{ATP} + x_{ADP})}{dt} = v_{-5} - v_5$$

These linear combinations are not coming out of the blue and we may try to be systematic in their construction. For instance let us look for all possible pool variables depending on intermediate time responses. We may write the differential equation as

$$\mathbf{l}^{T} \frac{\frac{d\mathbf{x}}{dt}}{\frac{d\mathbf{x}}{dt}} = \mathbf{l}^{T} S \mathbf{v}$$

In our case

$$\mathbf{v} = \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_{-5} \end{pmatrix} \begin{array}{c} intermediate \\ slow \\ fast \\ fa$$

So, we must find linear combinations l such that

$$\mathbf{l}^T S = (\cdot, \cdot, 0, 0, 0, 0)$$

That is we have the constraints

$$\mathbf{l}^T \mathbf{s}_3 = \mathbf{l}^T \mathbf{s}_4 = \mathbf{l}^T \mathbf{s}_5 = \mathbf{l}^T \mathbf{s}_6 = 0$$

where \mathbf{s}_i is the *i*-th column of *S*. We may write the corresponding linear equation system

$$egin{pmatrix} \mathbf{s}_1^3 \ \mathbf{s}_4^T \ \mathbf{s}_5^T \ \mathbf{s}_6^T \end{pmatrix} \mathbf{l} = \mathbf{0}$$

The solution of this equation system are all vectors of the form

$$\mathbf{l} = (2l_2, l_2, 0)$$

In particular, (2, 1, 0), that is 2ATP + ADP as analyzed in the example above is the only linear combination of this kind.

<u>Time scales</u>

Given a linear differential equation system with constant coefficients (this is always available if we linearize non-linear systems through the Jacobian)

$$\frac{d\mathbf{x}}{dt} = J\mathbf{x}$$

its general solution (of the linearized system) is of the form

$$\mathbf{x} = \sum_{i} c_i e^{\lambda_i t} \mathbf{v}_i$$



Figure 4: Depending on the module of the real part of the eigenvalues, remind that eigenvalues may be complex, we have faster (large module) or slower processes (small module). We expect all real parts to be negative if the system is stable.

where λ_i is an eigenvalue of J and \mathbf{v}_i its corresponding eigenvector. The constants c_i depend on the initial conditions. The eigenvalues of J determine the different speed processes (the larger its real part in module, the faster the process is; see Fig. 4).

Constant-volume assumption

Consider the concentration of a given compound in a cell, x, and the volume of that cell, V. The total amount of that substance in the cell is given by

M = xV

Its derivative is

$$\frac{dM}{dt} = \frac{dx}{dt}V + x\frac{dV}{dt}$$

If the volume of the cell is constant, then $\frac{dV}{dt} = 0$, and

$$\frac{dx}{dt} = \frac{1}{V}\frac{dM}{dt}$$

However, there are physiological conditions (e.g., cell division) where the assumption of constant volume is clearly incorrect. Apart from cell division, the volume in the cell compartments tend to fluctuate over time. However, not many models include these fluctuations due to the more complex mathematical analysis.

Osmotic balance

Cells normally have semipermeable membranes meaning that water (solvent) and some small molecules can diffuse freely through the membrane but large molecules (solute) cannot. In a cell, osmotic pressure inside and outside the cell are normally balanced, $\Pi_{inside} = \Pi_{outside}$. This puts a constraint on the concentrations inside and outside the cell, the sum of all concentrations must be constant:

$$RT \sum_{i} x_{i,inside} = RT \sum_{i} x_{i,outside}$$
$$\sum_{i} x_{i,inside} = \sum_{i} x_{i,outside}$$

This is particularly important for molecules that split or merge (remind that the concentration is defined as the number of molecules per unit volume, for instance molar concentration is $x = \frac{N}{N_A V}$ where N is the number of solute molecules, N_A is Avogadro's number and V the volume in liters).

Charge balance

Molecules may be neutral, positively or negatively charged (even their charge depends on the pH of their medium). At a given compartment it is standard to assume that the net charge is zero, this condition is called electroneutrality. If the charge of the *i*-th compound is z_i , another constraint on the concentrations is

$$\sum_{i} z_i x_i = 0$$

3 Palsson. Chap. 3. Dynamic simulation: the basic procedure

Practical exercise.

4 Palsson. Chap. 4. Chemical reactions

<u>The reversible linear reaction.</u> Consider the reversible reaction

$$A \xrightarrow[v_{BA}]{v_{BA}} B$$

The stoichiometric matrix is

$$S = \begin{pmatrix} -1 & 1\\ 1 & -1 \end{pmatrix}$$

and the velocity vector

$$\mathbf{v} = \begin{pmatrix} k_{AB} x_A \\ k_{BA} x_B \end{pmatrix}$$

. The only time invariant of this stoichiometric matrix is given by

$$x_A + x_B = ct$$

which simply reflects the fact that A is converted to B and viceversa.

Another interesting variable is defined by the net reaction velocity:

$$v_{nt} = v_{AB} - v_{BA} = k_{AB}x_A - k_{BA}x_B = k_{AB}\left(x_A - \frac{x_B}{K_{AB}}\right)$$

where $K_{AB} = \frac{k_{BA}}{k_{AB}} = \frac{x_B(equilibrium)}{x_A(equilibrium)}$. The term $x_A - \frac{x_B}{K_{AB}}$ measures the distance from the equilibrium state. So, we may perform a change of variables to

$$\begin{split} \tilde{\mathbf{x}} &= \begin{pmatrix} 1 & 1 \\ 1 & -\frac{1}{K_{AB}} \end{pmatrix} \mathbf{x} = P \mathbf{x} \Rightarrow \mathbf{x} = \frac{1}{K_{AB}+1} \begin{pmatrix} K_{AB} & 1 \\ 1 & -1 \end{pmatrix} \tilde{\mathbf{x}} = \begin{pmatrix} \frac{K_{AB}\tilde{x}_{1}+\tilde{x}_{2}}{K_{AB}+1} \\ \frac{\tilde{x}_{1}-\tilde{x}_{2}}{K_{AB}+1} \end{pmatrix} \\ \frac{d\tilde{\mathbf{x}}}{dt} &= P \frac{d\mathbf{x}}{dt} = P S \mathbf{v} = \begin{pmatrix} 1 & 1 \\ 1 & -\frac{1}{K_{AB}} \end{pmatrix} \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix} \begin{pmatrix} k_{AB} \frac{K_{AB}\tilde{x}_{1}+\tilde{x}_{2}}{K_{AB}+1} \\ k_{BA} \frac{\tilde{x}_{1}-\tilde{x}_{2}}{K_{AB}+1} \end{pmatrix} \\ &= (K_{AB}+1) \begin{pmatrix} 0 & 0 \\ -1 & 1 \end{pmatrix} \begin{pmatrix} k_{AB} \frac{K_{AB}\tilde{x}_{1}+\tilde{x}_{2}}{K_{AB}+1} \\ k_{BA} \frac{\tilde{x}_{1}-\tilde{x}_{2}}{K_{AB}+1} \end{pmatrix} \\ &= \begin{pmatrix} 0 & 0 \\ -1 & 1 \end{pmatrix} \begin{pmatrix} k_{AB} (K_{AB}\tilde{x}_{1}+\tilde{x}_{2}) \\ k_{BA} (\tilde{x}_{1}-\tilde{x}_{2}) \end{pmatrix} \\ &= \begin{pmatrix} 0 \\ -(k_{AB}+k_{BA})\tilde{x}_{2} \end{pmatrix} \end{split}$$

The first equation in this differential equation system states that \tilde{x}_1 is time invariant, the second one states that the equilibrium is restored with a time constant equal to $\tau_2 = \frac{1}{k_{AB}+k_{BA}}$.

Consider now the reaction

$$A^+B \xrightarrow[v_{r_{-1}}]{v_{r_{-1}}} C$$

The stoichiometric matrix is given by

$$S = \begin{pmatrix} -1 & 1\\ -1 & 1\\ 1 & -1 \end{pmatrix}$$

and the reaction velocity vector is

$$\mathbf{v} = \begin{pmatrix} v_1 \\ v_{-1} \end{pmatrix} = \begin{pmatrix} k_1 x_A x_B \\ k_{-1} x_C \end{pmatrix}$$

The reaction rate is defined as

$$\frac{dx_C}{dt} = -\frac{dx_A}{dt} = -\frac{dx_B}{dt} = k_1 x_A x_B - k_{-1} x_C = k_1 \left(x_A x_B - \frac{x_C}{K_1} \right)$$

where $K_1 = \frac{k_{-1}}{k_1}$. The reaction is at equilibrium when

$$\frac{dx_C}{dt} = 0 \Rightarrow x_C = K_1 x_A x_B$$

The left null space of S is formed by all vectors of the form $(l_3 - l_2, l_2, l_3)$. A possible basis of this subspace is $\{(1, 0, 1), (0, 1, 1)\}$. The corresponding time invariants are

$$\begin{array}{rcl} x_A + x_C &=& ct \\ x_B + x_C &=& ct \end{array}$$

As we did in the case of the reversible linear reaction, we could define three new variables, and study the system with these new variables

$$\tilde{\mathbf{x}} = \begin{pmatrix} x_A + x_C \\ x_B + x_C \\ x_A x_B - \frac{x_C}{K_1} \end{pmatrix}$$

However, this is not so useful now because the last variable, \tilde{x}_3 depends non-linearly on x_A and x_B .

Instead we may expand the right-hand side of the differential equation as a multivariate polynomial

$$\frac{d\mathbf{x}}{dt} = \begin{pmatrix} -k_1 x_A x_B + k_{-1} x_C \\ -k_1 x_A x_B + k_{-1} x_C \\ k_1 x_A x_B - k_{-1} x_C \end{pmatrix} = \begin{pmatrix} k_{-1} x_C \\ k_{-1} x_C \\ -k_{-1} x_C \end{pmatrix} + \begin{pmatrix} -k_1 x_A x_B \\ -k_1 x_A x_B \\ k_1 x_A x_B \end{pmatrix}$$

ODE linearization.

Although, this is not strictly needed because computer simulations can easily handle non-linearities as we simulate the time evolution of the compound concentrations, we may want to linearize a ODE system to better understand its local properties (for instance the structure of its initial conditions and its equilibrium). Let us assume that we know the initial conditions

$$x_A(0) = 3, x_B(0) = 2, x_C(0) = 0$$

and we know $k_1 = k_{-1} = K_1 = 1$. Our equation system is

$$\frac{d\mathbf{x}}{dt} = \begin{pmatrix} -k_1 x_A x_B + k_{-1} x_C \\ -k_1 x_A x_B + k_{-1} x_C \\ k_1 x_A x_B - k_{-1} x_C \end{pmatrix}$$

For a generic ODE system

$$\frac{d\mathbf{x}}{dt} = \mathbf{f}(\mathbf{x})$$

we may compute the first derivative of \mathbf{f} (the Jacobian)

$$J_{\mathbf{f}}(\mathbf{x}) = \frac{d\mathbf{f}(\mathbf{x})}{d\mathbf{x}} = \begin{pmatrix} \frac{\partial f_1}{\partial x_A} & \frac{\partial f_1}{\partial x_B} & \frac{\partial f_1}{\partial x_C} \\ \frac{\partial f_2}{\partial x_A} & \frac{\partial f_2}{\partial x_B} & \frac{\partial f_2}{\partial x_C} \\ \frac{\partial f_3}{\partial x_A} & \frac{\partial f_3}{\partial x_B} & \frac{\partial f_3}{\partial x_C} \end{pmatrix} (\mathbf{x})$$

and the ODE can be approximated by

$$\frac{d\mathbf{x}}{dt} = J_{\mathbf{f}}(\mathbf{x}_0)\mathbf{x}$$

where \mathbf{x}_0 is the point about which the linearization is performed.

In our particular case

$$J_{\mathbf{f}}(\mathbf{x}) = \begin{pmatrix} -k_1 x_B & -k_1 x_A & k_{-1} \\ -k_1 x_B & -k_1 x_A & k_{-1} \\ k_1 x_B & k_1 x_A & -k_{-1} \end{pmatrix} \Rightarrow J_{\mathbf{f}}(3,2,0) = \begin{pmatrix} -2 & -3 & 1 \\ -2 & -3 & 1 \\ 2 & 3 & -1 \end{pmatrix}$$

The eigenvalues of this matrix are -6 and 0 (twice). There is a single degree of freedom (only 1 eigenvalue is different from 0), and it behaves as a decaying process whose time constant is $\tau = \frac{1}{6}$.

The equilibrium is achieved for $x_{A,eq}, x_{B,eq}, x_{C,eq}$ such that

$$3 = x_A(0) + x_C(0) = x_{A,eq} + x_{C,eq}$$

$$2 = x_B(0) + x_C(0) = x_{B,eq} + x_{C,eq}$$

$$x_{A,eq} x_{B,eq} = x_{C,eq}$$

$$x_{A,eq} = \sqrt{3}$$

$$x_{B,eq} = \sqrt{3} - 1$$

$$x_{C,eq} = 3 - \sqrt{3}$$

The velocity of the reaction at these concentrations is

$$\mathbf{v}(\sqrt{3},\sqrt{3}-1,3-\sqrt{3}) = (0,0,0)^T$$

that is, the equilibrium is a critical point. The Jacobian at this point becomes

$$J_{\mathbf{f}}(\sqrt{3},\sqrt{3}-1,3-\sqrt{3}) = \begin{pmatrix} 1-\sqrt{3} & -\sqrt{3} & 1\\ 1-\sqrt{3} & -\sqrt{3} & 1\\ \sqrt{3}-1 & \sqrt{3} & -1 \end{pmatrix}$$

whose eigenvalues are -3.4641 and 0 (twice). The process arrives at equilibrium following a trajectory with time constant $\tau = \frac{1}{3.4641}$. Since the non-zero eigenvalues are real and negative, the critical point is stable.

5 Palsson. Chap. 5. Enzyme kinetics

Hill kinetics.

Michaelis-Menten model is the simplest enzymatic reaction model (the enzyme binds its substrate and then releases the product).

$$S + E \xrightarrow{k} E + P$$

Hill's model is a little bit more complicated and the enzyme, E, can be found in an inactive form, X. This inhibition is performed by an inhibitor, I

$$E + \nu I \xrightarrow[k_i^-]{k_i^-} X$$

 ν is normally found to be larger than 1 (even, non-integers), and a more realistic explanation is given by the symmetry model below. The mass action equations of the two coupled equations are

$$\begin{array}{rcl} \frac{dx_S}{dt} &=& -v_1\\ \frac{dx_E}{dt} &=& -v_2 + v_3\\ \frac{dx_P}{dt} &=& v_1\\ \frac{dx_I}{dt} &=& -\nu(v_2 - v_3)\\ \frac{dx_X}{dt} &=& v_2 - v_3 \end{array}$$

with

$$\begin{aligned} w_1 &= k x_S x_E \\ w_2 &= k_i^+ x_I^\nu x_E \\ w_3 &= k_i^- x_X \end{aligned}$$

The stoichiometric matrix is given by

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

and its left null space provides the following conservation quantities

$$\begin{array}{rcl} x_S + x_P &=& x_S(0) \\ x_E + x_X &=& x_E(0) \\ x_I + \nu x_X &=& x_I(0) \end{array}$$

The 3 conservation quantities allow reducing the 5 differential equations to just 2 of them. Let us choose S and E, then the system can be simulated by

$$\frac{dx_S}{dt} = -kx_Sx_E
\frac{dx_E}{dt} = -k_i^+ x_I^\nu x_E + k_i^- x_X
x_P = x_S(0) - x_S
x_X = x_E(0) - x_E
x_I = x_I(0) - \nu x_X$$

Simplified Hill kinetics.

If the inhibition of the enzyme is a fast process, then the chemical reaction $E + \nu I \xrightarrow[k_i^-]{k_i^-} X$ is at equilibrium (Quasi-Equilibrium Assumption, QEA), implying

$$v_2 = v_3 \Rightarrow \begin{cases} k_i^+ x_I^\nu x_E = k_i^- x_X \Rightarrow x_X = \left(\frac{k_i^+}{k_i^-}\right) x_I^\nu x_E = \left(\frac{x_I}{K_i}\right)^\nu x_E \\ \frac{dx_E}{dt} = \frac{dx_I}{dt} = \frac{dx_X}{dt} = 0 \end{cases}$$

where $K_i = \left(\frac{k_i^-}{k_i^+}\right)^{\frac{1}{\nu}}$ is a "per-site" dissociation constant. We may further exploit the equilibrium of the enzyme to calculate the concentration of active

enzyme

$$x_E = x_E(0) - x_X = x_E(0) - \left(\frac{x_I}{K_i}\right)^{\nu} x_E \Rightarrow x_E = \frac{x_E(0)}{1 + \left(\frac{x_I}{K_i}\right)^{\nu}}$$

The main reaction velocity, v_1 , can be calculated as

$$v_1 = kx_E x_S = \frac{kx_E(0)x_S}{1 + \left(\frac{x_L}{K_i}\right)^{\nu}} = \frac{v_{max}(x_S)}{1 + \left(\frac{x_L}{K_i}\right)^{\nu}}$$

where $v_{max}(x_S) = kx_E(0)x_S$. The most famous version of Hill's kinetics is

$$v = \frac{v_{max} x_L^{\nu}}{K_{0.5}^{\nu} + x_L^{\nu}}$$

However, this is the solution to a different problem (a ligand that binds multiple sites of a substrate). Before blindly applying a known solution we need to verify if the problem solved and the assumptions are the same to our problem.

Simmetry model.

Let us consider the inhibition reaction

$$\mathrm{E} + \nu \mathrm{I} \xrightarrow[]{\mathbf{k_i}^+} \mathrm{X}$$

In reality it is rare that the ν molecules of the inhibitor bind simultaneously to the enzyme. In practice, this reaction will take place in sequential steps:

$$\begin{split} & E + I \underbrace{ \xrightarrow{\nu k_i^+}}_{k_i^-} X_1 \\ & X_1 + I \underbrace{ \xrightarrow{(\nu-1) k_i^+}}_{2 k_i^-} X_2 \\ & X_2 + I \underbrace{ \xrightarrow{(\nu-2) k_i^+}}_{\dots} X_3 \\ & \dots \\ & X_{\nu-1} + I \underbrace{ \xrightarrow{k_i^+}}_{\nu k_i^-} X \end{split}$$

We may now write the mass action equations

$$\begin{aligned} \frac{dx_S}{dt} &= -v_1 = -kx_S x_E \\ \frac{dx_P}{dt} &= v_1 = kx_S x_E \\ \frac{dx_E}{dt} &= -v_2 + v_3 = -\nu k_i^+ x_I x_E + k_i^- x_{X_1} \\ \frac{dx_{X_1}}{dt} &= v_2 - v_3 - v_4 + v_5 = \nu k_i^+ x_I x_E - k_i^- x_{X_1} - (\nu - 1)k_i^+ x_I x_{X_1} + 2k_i^- x_{X_2} \\ \frac{dx_{X_2}}{dt} &= v_4 - v_5 - v_6 + v_7 = (\nu - 1)k_i^+ x_I x_{X_1} - k_i^- x_{X_2} - (\nu - 2)k_i^+ x_I x_{X_2} + 3k_i^- x_{X_3} \\ & \dots \end{aligned}$$

$$\begin{aligned} \frac{dx_{X_{\nu-1}}}{dt} &= v_{2(\nu-2)+2} - v_{2(\nu-2)+3} - v_{2(\nu-1)+2} + v_{2(\nu-1)+3} \\ &= 2k_i^+ x_I x_{X_{\nu-2}} - (\nu - 1)k_i^- x_{X_{\nu-1}} - k_i^+ x_I x_{X_{\nu-1}} + \nu k_i^- x_X \\ \frac{dx_X}{dt} &= v_{2(\nu-1)+2} - v_{2(\nu-1)+3} = k_i^+ x_I x_{X_{\nu-1}} - \nu k_i^- x_X \\ \frac{dx_I}{dt} &= \sum_{k=1}^{\nu} (-v_{2(\nu-1)+2} + v_{2(\nu-1)+3}) \end{aligned}$$

The time invariants of this system are

$$\begin{aligned} x_E(0) &= x_E + x_{X_1} + x_{X_2} + \dots + x_{\nu-1} + x_X \\ x_I(0) &= x_I + x_{X_1} + 2x_{X_2} + \dots + (\nu - 1)x_{\nu-1} + \nu x_X \\ x_S(0) &= x_S + x_P \end{aligned}$$

Nondimensionalization.

Nondimensionalization is a technique that helps to parametrize differential equations through a process called *scaling*. This technique stems from Buck-ingham's π theorem. This theorem, loosely speaking, states that if an equation uses n variables and k physical units, then the equation can be written using only n - k dimensionless parameters. Scaling is normally performed using 3 steps that we will first illustrate on an exponential decay equation

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

Step 1. List all variables and constants along with their physical units

Variables	Units	Parameters	Units
t	s	k	s^{-1}
x	$mole \cdot L^{-1}$	x_0	$mole \cdot L^{-1}$

Step 2. For each variable define a new dimensionless variable by multiplying/dividing that variable by the appropriate parameters.

Variables	Units	Parameters	Units
t	s	k	s^{-1}
x	$mole \cdot L^{-1}$	x_0	$mole \cdot L^{-1}$
$\tau = kt$	-		
$\chi = \frac{x}{x_0}$	-		

Step 3. Rewrite the differential equation using the new variables. For this we need first to calculate the derivatives

$$\frac{dx}{dt} = x_0 \frac{d\chi}{dt} = x_0 \frac{d\chi}{d\tau} \frac{d\tau}{dt} = k x_0 \frac{d\chi}{d\tau}$$

and then transform the differential equation

$$kx_0 \frac{d\chi}{d\tau} = -kx_0\chi \Rightarrow \frac{d\chi}{d\tau} = -\chi$$

Note that none of the terms in this equation has physical units, therefore, the name nondimensionalization for this procedure. Finally, we solve the equation and undo the change of variables

$$\frac{d\chi}{d\tau} = -\chi \Rightarrow \chi(\tau) = e^{-\tau} \Rightarrow \frac{x(t)}{x_0} = e^{-kt} \Rightarrow x(t) = x_0 e^{-kt}$$

Nondimensionalization of Hill kinetics.

Let us repeat this procedure for a more complicated system. Let us take as an example Hill kinetics which we reproduce here for convenience

$$\frac{\frac{dx_S}{dt}}{\frac{dx_E}{dt}} = -kx_Sx_E$$

$$\frac{\frac{dx_E}{dt}}{\frac{dx_P}{dt}} = -k_i^+ x_I^\nu x_E + k_i^- x_X$$

$$\frac{\frac{dx_P}{dt}}{\frac{dt}{dt}} = kx_Sx_E$$

$$\frac{\frac{dx_X}{dt}}{\frac{dt_X}{dt}} = -\nu(k_i^+ x_I^\nu x_E - k_i^- x_X)$$

Step 1.

Variables	Units	Parameters	Units
t	s	k	$L \cdot mole^{-1} \cdot s^{-1}$
x_S	$mole \cdot L^{-1}$	s_0	$mole \cdot L^{-1}$
x_E	mole $\cdot L^{-1}$	e_0	$mole \cdot L^{-1}$
x_P	$mole \cdot L^{-1}$	p_0	$mole \cdot L^{-1}$
x_I	$mole \cdot L^{-1}$	i_0	$mole \cdot L^{-1}$
x_X	$mole \cdot L^{-1}$	x_0	$mole \cdot L^{-1}$
		k_i^+	$L^{\nu} \cdot mole^{-\nu} \cdot s^{-1}$
		k_i^-	s^{-1}

Step ,	2.
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Variables	Units	Parameters	\mathbf{Units}
t	s	k	$L \cdot mole^{-1} \cdot s^{-1}$
x_S	$mole \cdot L^{-1}$	s_0	$mole \cdot L^{-1}$
x_E	$mole \cdot L^{-1}$	e_0	$mole \cdot L^{-1}$
x_P	$mole \cdot L^{-1}$	p_0	$mole \cdot L^{-1}$
x_I	mole $\cdot L^{-1}$	i_0	$mole \cdot L^{-1}$
x_X	$mole \cdot L^{-1}$	x_0	$mole \cdot L^{-1}$
		k_i^+	$L^{\nu} \cdot mole^{-\nu} \cdot s^{-1}$
		k_i^-	s^{-1}
$\tau = k s_0 t$	-		
$\chi_S = \frac{x_S}{s_0}$	-		
$\chi_E = \frac{x_E}{e_0}$	-		
$\chi_P = \frac{x_P}{p_0}$	-		
$\chi_I = \frac{\hat{x}_I}{i_0}$	-		
$\chi_X = \frac{x_X}{x_0}$	-		

Step 3. We first calculate the needed derivatives

$$\begin{array}{rcl} \frac{dx_S}{dt} &=& s_0 \frac{d\chi_S}{dt} = s_0 \frac{d\chi_S}{d\tau} \frac{d\tau}{dt} = k s_0^2 \frac{d\chi_S}{d\tau} \\ \frac{dx_E}{dt} &=& k s_0 e_0 \frac{d\chi_E}{d\tau} \\ \frac{dx_P}{dt} &=& k s_0 p_0 \frac{d\chi_P}{d\tau} \\ \frac{dx_I}{dt} &=& k s_0 i_0 \frac{d\chi_I}{d\tau} \\ \frac{dx_X}{dt} &=& k s_0 x_0 \frac{d\chi_X}{d\tau} \end{array}$$

We now rewrite the differential equations in terms of the new variables

$$ks_{0}^{2}\frac{d\chi_{S}}{d\tau} = -ks_{0}\chi_{S}e_{0}\chi_{E}$$

$$ks_{0}e_{0}\frac{d\chi_{E}}{d\tau} = -k_{i}^{+}(i_{0}\chi_{I})^{\nu}e_{0}\chi_{E} + k_{i}^{-}x_{0}\chi_{X}$$

$$ks_{0}p_{0}\frac{d\chi_{P}}{d\tau} = ks_{0}\chi_{S}e_{0}\chi_{E}$$

$$ks_{0}i_{0}\frac{d\chi_{I}}{d\tau} = -\nu(k_{i}^{+}(i_{0}\chi_{I})^{\nu}e_{0}\chi_{E} - k_{i}^{-}x_{0}\chi_{X})$$

$$ks_{0}x_{0}\frac{d\chi_{X}}{d\tau} = k_{i}^{+}(i_{0}\chi_{I})^{\nu}e_{0}\chi_{E} - k_{i}^{-}x_{0}\chi_{X}$$

and rearrange them

$$\frac{d\chi_S}{d\tau} = -\frac{e_0}{s_0} \chi_S \chi_E$$

$$\frac{d\chi_E}{d\tau} = -\frac{k_i^+ i_0^{\nu}}{ks_0} \chi_I^{\nu} \chi_E + \frac{k_i^- x_0}{ks_0 e_0} \chi_X$$

$$\frac{d\chi_P}{d\tau} = \frac{e_0}{p_0} \chi_S \chi_E$$

$$\frac{d\chi_I}{d\tau} = -\nu \frac{k_i^+ i_0^{\nu-1} e_0}{ks_0} \chi_I^{\nu} \chi_E + \nu \frac{k_i^- x_0}{ks_0 i_0} \chi_X$$

$$\frac{d\chi_X}{d\tau} = \frac{k_i^+ i_0^{\nu} e_0}{ks_0 x_0} \chi_I^{\nu} \chi_E - \frac{k_i^-}{ks_0} \chi_X$$

Note that all terms in the differential equation are adimensional as can be easily verified.

6 Palsson. Chap. 6. Open systems

Reversible reaction in an open environment. Let us consider the reaction

$$\xrightarrow{b_1} x_1 \xrightarrow[v_{-1}]{v_1} x_2 \xrightarrow{b_2} x_2$$

where b_1 is a constant input flow and $b_2 = k_2 x_2$ is an output flow. We may write the system as

$$\begin{pmatrix} \frac{dx_1}{dt} \\ \frac{dx_2}{dt} \end{pmatrix} = \begin{pmatrix} 1 & -1 & 1 & 0 \\ 0 & 1 & -1 & -1 \end{pmatrix} \begin{pmatrix} b_1 \\ k_1 x_1 \\ k_{-1} x_2 \\ k_2 x_2 \end{pmatrix}$$

The left null space is $\{0\}$ meaning that there is no time invariant.

The steady state $S\mathbf{v}_{ss} = \mathbf{0}$ is formed by all vectors in the right null space, which is spanned by the vectors (1, 1, 0, 1) and (0, 1, 1, 0). The former is the forward path thr ugh the system $(b_1 \rightarrow v_1 \rightarrow b_2)$. The latter corresponds to the reversible reaction $(v_1 \leftrightarrow v_{-1})$. All steady states are a non-negative combination f these two vectors:

$$\mathbf{v}_{ss} = (b_1, k_1 x_{1,ss}, k_{-1} x_{2,ss}, k_2 x_{2,ss}) = a(1, 1, 0, 1) + b(0, 1, 1, 0) \quad a, b \ge 0$$

We may also calculate the steady state concentrations. If we analyze the condition of the steady state, we have:

$$S\mathbf{v}_{ss} = \mathbf{0} \Rightarrow \begin{pmatrix} b_1 - k_1 x_{1,ss} + k_{-1} x_{2,ss} \\ k_1 x_{1,ss} - k_{-1} x_{2,ss} - k_2 x_{2,ss} \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \end{pmatrix} \Rightarrow \begin{pmatrix} x_{1,ss} \\ x_{2,ss} \end{pmatrix} = \frac{b_1}{k_2} \begin{pmatrix} \frac{k_2 + k_{-1}}{k_1} \\ 1 \end{pmatrix}$$

Substituting $x_{1,ss}$ and $x_{2,ss}$ into \mathbf{v}_{ss} , we get

$$\mathbf{v}_{ss} = b_1 \left(1, 1 + \frac{k_{-1}}{k_2}, \frac{k_{-1}}{k_2}, 1 \right)$$

Note that by definition, at the steady state the input flow is equal to the output flow, $b_1 = b_2$. This steady state is achieved for

$$a = b_1 \quad b = \frac{b_1 k_{-1}}{k_2}$$

In the absence of external flows (input and output), the system is at equilibrium when

$$\frac{x_{2,eq}}{x_{1,eq}} = \frac{k_1}{k_{-1}}$$

We may calculate the ratio between the relationship between x_2 and x_1 at equilibrium and at the steady state:

$$\frac{\Gamma}{K_{eq}} = \frac{\frac{x_{2,ss}}{x_{1,ss}}}{\frac{x_{2,eq}}{x_{1,eq}}} = \frac{\frac{\frac{1}{k_2+k_{-1}}}{k_1}}{\frac{k_1}{k_{-1}}} = \frac{1}{1+\frac{k_2}{k_{-1}}}$$

If $k_2 \ll k_{-1}$ then $\frac{\Gamma}{K_{eq}} \approx 1$, meaning that if the amount of x_2 that escapes the system is much smaller than the amount of x_2 that goes back to x_1 , then the open system behaves like the closed system.

Michaelis-Menten kinetics in an open environment.

Let us analyze the Michaelis-Menten kinetics of Eq. 1 when the substrate arrives with an input flow b_1 and the product leaves at a flow $v_3 = k_3 x_P$:

$$\xrightarrow{b_1} S + E \xleftarrow{k_f}{k_b} SE \xrightarrow{k_{cat}} E + P \xrightarrow{v_3}$$

We may write the differential equations associated to these reactions

$$\begin{array}{rcl} \frac{dx_S}{dt} &=& b_1 - k_f x_S x_E + k_b x_{SE} \\ \frac{dx_E}{dt} &=& -k_f x_S x_E + k_b x_{SE} + k_{cat} x_{SE} \\ \frac{dx_{SE}}{dt} &=& k_f x_S x_E - k_b x_{SE} - k_{cat} x_{SE} \\ \frac{dx_P}{dt} &=& k_{cat} x_{SE} - k_3 x_P \end{array}$$

Or equivalently

$$\begin{pmatrix} \frac{dx_S}{dt} \\ \frac{dx_E}{dt} \\ \frac{dx_SE}{dt} \\ \frac{dx_P}{dt} \end{pmatrix} = \begin{pmatrix} 1 & -1 & 1 & 0 & 0 \\ 0 & -1 & 1 & 1 & 0 \\ 0 & 1 & -1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{pmatrix} \begin{pmatrix} b_1 \\ k_f x_E x_S \\ k_b x_{SE} \\ k_{cat} x_{SE} \\ k_3 x_P \end{pmatrix}$$

There is only one time invariant (left-null space) that is $x_E + x_{SE} = x_E(0)$. Let us calculate now the steady state fluxes. The null space of S is spanned by the vectors (1, 1, 0, 1, 1) and (0, 1, 1, 0, 0) that correspond to a pathway through the system $(v_1 \rightarrow k_f \rightarrow k_{cat} \rightarrow k_3)$ and the reversible reaction $(v_f \leftrightarrow v_b)$. In this way,

$$\mathbf{v}_{ss} = (b_1, k_f x_{E,ss} x_{S,ss}, k_b x_{SE,ss}, k_{cat} x_{SE,ss}, k_3 x_{P,ss}) = a(1, 1, 0, 1, 1) + b(0, 1, 1, 0, 0)$$

If we solve the equation

If we solve the equation

$$S\mathbf{v}_{ss} = \mathbf{0} \Rightarrow \begin{pmatrix} x_{S,ss} \\ x_{E,ss} \\ x_{SE,ss} \\ x_{P,ss} \end{pmatrix} = \begin{pmatrix} \frac{k_{cat}}{k_f} \frac{k_b/k_{-1}+1}{x_E(0)k_{cat}/b_{1}-1} \\ x_E(0) - \frac{b_1}{k_{cat}} \\ \frac{b_1}{k_{cat}} \\ \frac{b_1}{k_3} \end{pmatrix}$$

The steady state fluxes are then

$$\mathbf{v}_{ss} = \begin{pmatrix} b_1 & b_1 \left(1 + \frac{k_b}{k_{cat}} \right), b_1 \frac{k_b}{k_{cat}}, b_1, b_1 \end{pmatrix}$$

That is

$$b_1 = v_f - v_b = v_{cat} = v_3$$

This steady state is achieved by $a = b_1$ and $b = b_1 \frac{k_b}{k_{cat}}$.

Palsson. Chap. 7. Orders of magnitude 7

To be prepared by students.

Chap. 8. Stoichiometric struc-Palsson. 8 ture

To be prepared by students.

9 Palsson. Chap. 9. Regulation as elementary phenomena

Let us consider the simple scheme

$$\xrightarrow{v_1(x)} X \xrightarrow{v_2(x)}$$

where the concentration of the metabolite X, x, influences the rates of its own formation, $v_1(x)$, and its degradation, $v_2(x)$. The dynamic mass balance is given by

$$\frac{dx}{dt} = v_1(x) - v_2(x)$$

We may linearize this equation as

$$\frac{dx}{dt} = \left(\frac{\partial v_1}{\partial x}(x_0) - \frac{\partial v_2}{\partial x}(x_0)\right) x = \lambda(x_0)x$$

where

$$\lambda(x_0) = \frac{\partial v_1}{\partial x}(x_0) - \frac{\partial v_2}{\partial x}(x_0)$$

is the "net" flux at a concentration x_0 of the metabolite.

We may encounter different regulatory situations:

• Unregulation: The metabolite is actually not regulated by itself:

$$\frac{\partial v_1}{\partial x}(x_0) = 0 \Rightarrow \lambda(x_0) < 0$$

• <u>Feedback inhibition</u>: If the formation of the metabolite is inhibited by itself, then

$$\frac{\partial v_1}{\partial x}(x_0) < 0$$

• <u>Feedback activation</u>: If the formation of the metabolite is activated by itself, then

$$\frac{\partial v_1}{\partial x}(x_0) > 0$$

• <u>Feedforward inhibition</u>: If the degradation of the metabolite is inhibited by itself, then $\frac{\partial v_2}{\partial x}(x_0)$ is reduced (although it is still positive). • <u>Feedforward activation</u>: If the degradation of the metabolite is activated by itself, then $\frac{\partial v_2}{\partial x}(x_0)$ is increased.

Feedback inhibition and feedforward activation stabilize the system, while feedback activation and feedforward inhibition may create instabilities and shift the cell to a totally different state.

We may express λ as

$$\lambda(x_0) = -\frac{\partial v_2}{\partial x}(x_0) \left(1 - \frac{\frac{\partial v_1}{\partial x}(x_0)}{\frac{\partial v_2}{\partial x}(x_0)} \right) = -\frac{\partial v_2}{\partial x}(x_0) \left(1 - a(x_0) \right)$$

If $a(x_0) < 1$, then the system is locally stable (around x_0). If $a(x_0) > 1$, then the system is locally unstable.

Local inhibition with Hill kinetics.

Let us show an example of the application of this theoretical framework to the case of local inhibition with Hill kinetics at the steady state. Let us consider

$$v_1(x) = \frac{v_{max}}{1 + \left(\frac{x}{K}\right)^2}$$
$$v_2(x) = kx$$

 v_1 is Hill-type equation with $\nu = 2$. The steady state is achieved when

$$\begin{aligned} \frac{v_1(x_{ss})}{\frac{v_{max}}{1 + \left(\frac{x}{K}\right)^2}} &= kx \\ \left(\frac{x_{ss}}{K}\right)^3 + \left(\frac{x_{ss}}{K}\right) - \frac{v_{max}}{kK} &= 0 \end{aligned}$$

This equation is of the form

$$\chi^3 + \chi - a = 0$$

with $a = \frac{v_{max}}{kK}$ and its solution is

$$x_{ss} = K\left(\frac{(9a + \sqrt{3}\sqrt{4 + 27a^2})^{1/3}}{2^{1/3}3^{2/3}} - \frac{(2/3)^{1/3}}{(9a + \sqrt{3}\sqrt{4 + 27a^2})^{1/3}}\right)$$

The linearization constant λ is

$$\lambda = -\frac{2K^2 v_{max} x_{ss}}{(K^2 + x_{ss}^2)^2} - k = -k \left(1 + \frac{2v_{max}}{Kk} \frac{\frac{x_{ss}}{K}}{\left(1 + \left(\frac{x_{ss}}{K}\right)^2\right)^2} \right)$$

Note that λ is always negative, meaning that at the steady state the system is stable. Depending on the values of v_{max} , k and K, if $x_{ss} \ll K$, then $\lambda \approx -k$ meaning that the input is almost unregulated. If $x_{ss} \gg K$, then $\lambda \approx -k$ meaning that the system is again almost unregulated. The function $\frac{x}{(1+x^2)^2}$ has a maximum at $x = \frac{1}{\sqrt{3}}$, in our case, $\frac{x_{ss}}{K} = \frac{1}{\sqrt{3}}$. The maximum of this function is $\frac{3\sqrt{3}}{16}$ so that around the steady state, the maximum achievable regulation is

$$\lambda = -k\left(1 + \frac{2v_{max}}{Kk}\frac{3\sqrt{3}}{16}\right)$$

<u>Local activation with Hill kinetics</u>. Let us consider the creation and degradation flows

$$\begin{cases} v_1(x) &= v_{max} \frac{1+\alpha\left(\frac{x}{K}\right)^{\nu}}{1+\left(\frac{x}{K}\right)^{\nu}} \\ v_2(x) &= kx \end{cases} \end{cases} \Rightarrow \frac{dx}{dt} = v_{max} \frac{1+\alpha\left(\frac{x}{K}\right)^{\nu}}{1+\left(\frac{x}{K}\right)^{\nu}} - kx$$

Let us nondimensionalize the equation with the change of variables

$$\begin{aligned} \tau &= kt \\ \chi &= \frac{x}{K} \end{aligned} \} \Rightarrow \frac{dx}{dt} = \frac{d(K\chi)}{d\tau} \frac{d\tau}{dt} = Kk \frac{d\chi}{d\tau} \end{aligned}$$

The differential equation becomes

$$\begin{array}{rcl} Kk\frac{d\chi}{d\tau} &=& v_{max}\frac{1+\alpha\chi^{\nu}}{1+\chi^{\nu}}-k(K\chi)\\ \frac{d\chi}{d\tau} &=& a\frac{1+\alpha\chi^{\nu}}{1+\chi^{\nu}}-\chi \end{array}$$

with $a = \frac{v_{max}}{Kk} > 0$. The steady state of this system is achieved when

$$a\frac{1+\alpha\chi_{ss}^{\nu}}{1+\chi_{ss}^{\nu}}-\chi_{ss}=0 \Rightarrow \chi_{ss}^{\nu+1}-a\alpha\chi_{ss}^{\nu}+\chi_{ss}-a=0$$

This is a polynomial of degree $\nu + 1$ and has $\nu + 1$ roots. Depending on the values of α and a many of them may be real, meaning that there are several steady states, note that only those roots $\chi_{ss} = \frac{x_{ss}}{K} > 0$ are biologically plausible. A necessary condition (see Palsson Chap. 9) for the existence of multiple steady states is

$$\alpha > \left(\frac{1+\nu}{1-\nu}\right)^2$$

(

The linearization of this nondimensional differential equation gives

$$\lambda = \frac{\nu a(\alpha - 1)\chi_{ss}^{\nu - 1}}{(1 + \chi_{ss}^{\nu})^2} - 1$$

Depending on the sign of λ at the different steady states, some of them will be unstable ($\lambda > 0$) and some other stable ($\lambda < 0$).

Feedback inhibition in pathways.

Consider the biosynthetic pathway represented in Fig. 5.



Figure 5: Biosynthetic pathway example.

A biosynthetic precursor x_1 is formed (at constant rate b_1) and degraded (at rate $v_0 = k_0 x_1$)

$$\xrightarrow{b_1} X_1 \xrightarrow{v_0}$$

If the enzyme X_6 is expressed, X_1 can be converted to X_2

$$X_1 + X_6 \xrightarrow{v_1} X_2 + X_6$$

which is followed by a series of reactions

$$X_2 \xrightarrow{v_2} X_3 \xrightarrow{v_3} X_4 \xrightarrow{v_4} X_5$$

The product X_5 is exported to another cell compartment at a rate v_5

$$X_5 \xrightarrow{v_5}$$

The end product X_5 binds to the enzyme X_6 inhibiting it in an inactive state

$$X_5 + X_6 \xrightarrow[v_{-6}]{v_{-6}} X_7$$

The differential equations that describe the dynamic behavior of this system are

$$\begin{array}{rclrcl} \frac{dx_1}{dt} &=& b_1 - v_0 - v_1 &=& b_1 - k_0 x_1 - k_1 x_6 x_1 \\ \frac{dx_2}{dt} &=& v_1 - v_2 &=& k_1 x_6 x_1 - k_2 x_2 \\ \frac{dx_3}{dt} &=& v_2 - v_3 &=& k_2 x_2 - k_3 x_3 \\ \frac{dx_4}{dt} &=& v_3 - v_4 &=& k_3 x_3 - k_4 x_4 \\ \frac{dx_5}{dt} &=& v_4 - v_5 - v_6 + v_{-6} &=& k_4 x_4 - k_5 x_5 - k_6 x_5 x_6 + k_{-6} x_7 \\ \frac{dx_6}{dt} &=& -v_6 + v_{-6} &=& -k_6 x_5 x_6 + k_{-6} x_7 \\ \frac{dx_7}{dt} &=& v_6 - v_{-6} &=& k_6 x_5 x_6 - k_{-6} x_7 \end{array}$$

The sum of the last two equations gives one of the time invariants

$$\frac{dx_6}{dt} + \frac{dx_7}{dt} = 0 = \frac{d(x_6 + x_7)}{dt} \Rightarrow x_6(t) + x_7(t) = e_T$$

where e_T is the total amount of the enzyme.

In the synthesis rate of the precursor, b_1 , increases by a factor 10, thanks to the negative feedback, the synthesis rate of the metabolic output does not increase that much (see Fig. 6).

Regulation of protein synthesis.

The end product may also regulate the amount of X_6 enzyme present (such that $x_6(t) + x_7(t)$ is no longer constant). We may model this by adding two terms to the dynamics of X_6 , one corresponding to its formation, controlled by X_5 , and another one for its degradation:

$$\frac{dx_6}{dt} = -v_6 + v_{-6} + v_7 - v_8 = -k_6 x_5 x_6 + k_{-6} x_7 + \frac{k_7}{1 + K_7 x_5} - k_8 x_6$$

Tight regulation of enzyme activity.

The mechanism above is not too effective in stabilizing the synthesis rate of the end product (see Palsson, Chap. 9). The reason is that regulated enzymes normally respond to a more complex model than two (active/inactive) states. The symmetry model allows this extra control of the enzyme through a series of binding sites. If the enzyme allows up to 4 X_5 molecules, then the extra reactions to consider are



Figure 6: Biosynthetic pathway response after 10x increase of precursor synthesis.

$$X_{6} + X_{5} \underbrace{\frac{4 k_{6}}{k_{-6}}}_{K_{7}} X_{7}$$
$$X_{7} + X_{5} \underbrace{\frac{3 k_{6}}{2 k_{-6}}}_{2 k_{-6}} X_{8}$$
$$X_{8} + X_{5} \underbrace{\frac{2 k_{6}}{3 k_{-6}}}_{3 k_{-6}} X_{9}$$
$$X_{9} + X_{5} \underbrace{\frac{k_{6}}{4 k_{-6}}}_{4 k_{-6}} X_{10}$$

The higher the number of binding sites for X_5 , the more effective is the negative feedback.