Chemical and enzyme kinetics

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Master en Bioinformatique et Modélisation

Contents

1 Definitions

1.1 Reaction rate

Consider the chemical reaction that transforms the substrates A and B into the products C and D:

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{C} + \mathrm{D} \tag{1}$$

The variation in time of the concentration of the substrates (A and B) and the products (C and D),

$$\frac{dA}{dt}, \frac{dB}{dt}, \frac{dC}{dt}, \text{ and } \frac{dD}{dt} \tag{2}$$

is determined by the rate at which the reaction proceeds.

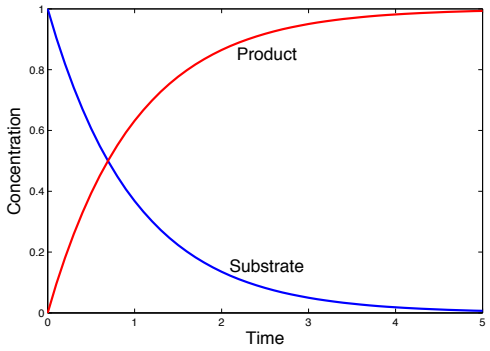


Figure 1: Time evolution of the concentration of the substrate and product.

For a chemical reaction to occur, the reacting species must collide, have sufficient energy and be well oriented. The number of collisions is proportional to the concentration of the reacting species. For the reaction (1), the rate law is given by the **mass action law**:

$$v = kAB \tag{3}$$

Not all collisions are reactive. The rate constant k accounts for the probability that the molecules are well oriented and have sufficient energy to react.

The variation in time of the concentration of the substrates and the products is given by

$$\frac{dA}{dt} = \frac{dB}{dt} = -kAB \text{ and } \frac{dC}{dt} = \frac{dD}{dt} = kAB \tag{4}$$

The sign in the right-hand side of these equations stands for the fact that, each time the reaction proceeds, one molecule (mole) of A (and B) disappears while one molecule (mole) of C (and D) appears.

More generally, for an (elementary) reaction in which m molecules of A react with p molecules of B and in which the products (C and D) do not affect the reaction rate:

$$\mathrm{mA} + \mathrm{pB} \rightarrow \mathrm{qC} + \mathrm{rD} \tag{5}$$

the rate law is:

$$v = kA^m B^p \tag{6}$$

Note that the sum $m + p$ is called the **order of a reaction**.

Consider now for example the following reaction:

$$3\text{A} + \text{B} \rightarrow \text{A} + \text{C} \tag{7}$$

According to Eq. (6), the rate of this reaction is:

$$v = kA^3 B \tag{8}$$

When we write the evolution of the concentration of A, we must take into consideration the fact that each time this reaction occurs, only two molecules of A are transformed (one is conserved). So, the variation of A is given by:

$$\frac{dA}{dt} = -2v = -2kA^3 B \tag{9}$$

The coefficient "2" is the balance for the species A in reaction (7) and the sign "-" stands because, globally, A is consumed. Since v must have the unit [concentration]/[time], the units of k depend on the order of the reaction.

In the general case, for a reaction in which for each n molecules (moles) of X transformed p molecules (moles) are recovered at the end:

$$\text{n X} + ... \rightarrow \text{p X} + ... \tag{10}$$

the evolution equation for the concentration of X is:

$$\frac{dX}{dt} = \eta_X v \text{ with } \eta_X = p - n \tag{11}$$

η_X is called the **stoechiometric coefficient** of compound X. This coefficient is positive if, globally, the species is produced ($p > n$) and negative if the species is consumed ($n > p$).

For example, for the following reaction:

$$\text{A} + 2\text{B} \rightarrow 3\text{A} + \text{C} \tag{12}$$

the stoechiometric coefficients of the different species are:

$$\eta_A = 3 - 1 = 2,\ \eta_B = 0 - 2 = -2,\ \eta_C = 1 - 0 = 1. \tag{13}$$

and the evolution equations are:

$$\frac{dA}{dt} = 2kAB^2,\ \frac{dB}{dt} = -2kAB^2,\ \frac{dC}{dt} = kAB^2 \tag{14}$$

1.2 Examples

1st-order kinetics

Consider the reaction of 1st-order:

$$\text{A} \rightarrow \text{A}^* \text{ (ex: conformational change of a molecule)} \tag{15}$$

or

$$\text{A} \rightarrow \text{B} + \text{C} \text{ (dissociation of a molecule into two molecules)} \tag{16}$$

By definition (eq 3), the rate of this reaction is

$$v = kA \tag{17}$$

and the time evolution of the concentration of the substrate A is:

$$\frac{dA}{dt} = -kA \tag{18}$$

After integration, we find:

$$A(t) = A_0 e^{-kt} \tag{19}$$

where A_0 is the initial concentration of substrate A ($A_0 = A(0)$).

We observe an exponential decrease of the concentration of A with time:

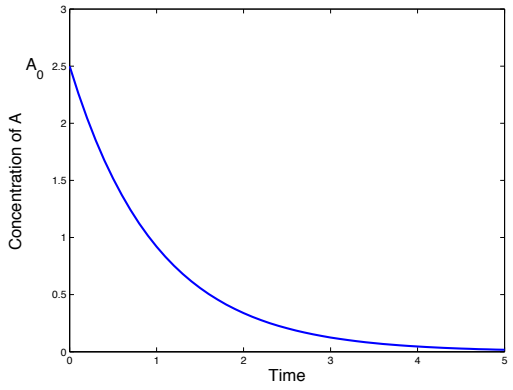


Figure 2: 1st-order kinetics: exponential decrease of the concentration of substrate A.

Remark: On the long run, A will converge to 0, i.e. A will be fully degraded. The half-life $\tau_{1/2}$ of A is defined by the time it takes for its concentration A to decrease from its initial value A_0 to half of this value, $A_0/2$:

$$\begin{aligned} \frac{A_0}{2} &= A_0 e^{-k\tau_{1/2}} \\ \tau_{1/2} &= \frac{\ln 2}{k} \end{aligned} \tag{20}$$

Note that $\tau_{1/2}$ does not depend on the initial value A_0.

2nd-order kinetics

Let's take now the reaction:

$$2\text{A} \rightarrow \text{B (2 molecules A fuse together to give one single molecule)} \tag{21}$$

or

$$2\text{A} \rightarrow \text{B+C (2 molecules A react together to give two different molecules)} \tag{22}$$

Its rate is:

$$v = kA^2 \tag{23}$$

and the time evolution of the substrate A is

$$\frac{dA}{dt} = -2kA^2 \tag{24}$$

After integration, we find:

$$A(t) = \frac{A_0}{1 + 2A_0kt} \tag{25}$$

where A_0 is the initial concentration of substrate A.

Here, we observe an hyperbolic decrease of the concentration of A with time:

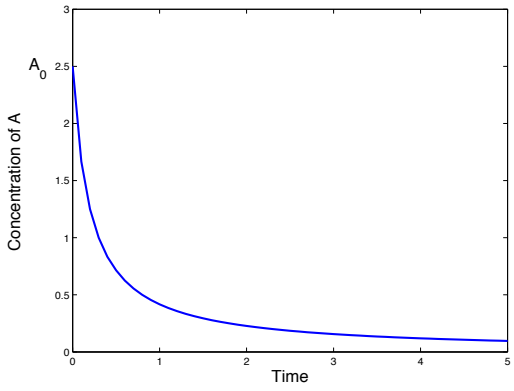


Figure 3: 2nd-order kinetics: hyperbolic decrease of the concentration of substrate A.

Reactions in series

Let's consider the following reactions in series:

$$\mathrm{A} \overset{k_1}{\rightarrow} \mathrm{B} \overset{k_2}{\rightarrow} \mathrm{C} \tag{26}$$

Evolution equations for the compounds A, B, and C write:

$$\frac{dA}{dt} = -k_1 A \tag{27}$$

$$\frac{dB}{dt} = k_1 A - k_2 B \tag{28}$$

$$\frac{dC}{dt} = k_2 B \tag{29}$$

Assuming that we start with $A(0) = A_0$ and $B(0) = C(0) = 0$, we find

$$A(t) = A_0 e^{-k_1 t} \qquad \text{(as before)} \tag{30}$$

and, after integration

$$B(t) = \frac{k_1 A_0}{k_2 - k_1} \left(e^{-k_1 t} - e^{-k_2 t} \right) \tag{31}$$

$$C(t) = A_0 \left(1 - \frac{1}{k_2 - k_1} \left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t} \right) \right) \tag{32}$$

The derivation is detailed in the Appendix.

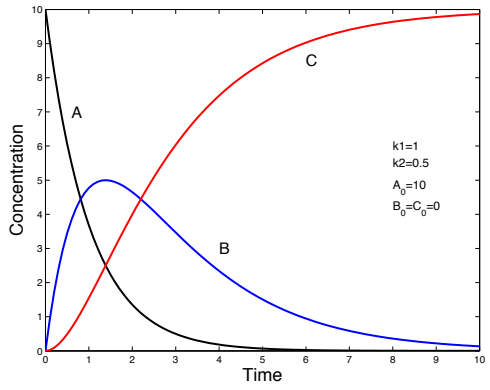


Figure 4: Kinetics of reactions in series.

Now we can consider the case where A is continuously supplied, so that A remains constant over time:

$$\overset{k_0}{\rightarrow} \text{A} \overset{k_1}{\rightarrow} \text{B} \overset{k_2}{\rightarrow} \text{C} \overset{k_3}{\rightarrow} \tag{33}$$

The evolution equations for the compound B is, with $A(t) = A_0 = k_0/k_1$:

$$\frac{dB}{dt} = k_1 A_0 - k_2 B \tag{34}$$

and the evolution of B is then given by:

$$B(t) = \frac{k_1 A_0 - (k_1 A_0 - k_2 B_0) e^{-k_2 t}}{k_2} \tag{35}$$

Now, on the long run and regardless of its initial concentration, B reaches a steady state:

$$B(\infty) = \frac{k_1 A_0}{k_2} \tag{36}$$

The derivation of the equation for $C(t)$ is left as an exercise.

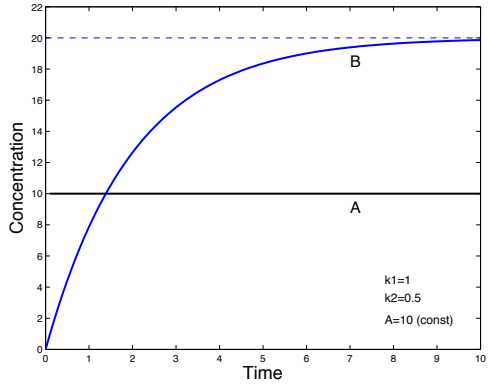


Figure 5: Kinetics of reactions in series with $A = A_0 =$ constant.

1.3 Systems of chemical reactions

We are usually interested by systems of coupled chemical reactions.

$$\begin{array}{rl} n_{11}\ X_1 + n_{21} X_2 + ... & \rightarrow p_{11}\ X_1 + p_{21}\ X_2 ... \\ n_{12}\ X_1 + n_{22} X_2 + ... & \rightarrow p_{12}\ X_1 + p_{22}\ X_2 ... \\ & ... \\ n_{1R}\ X_1 + n_{2R} X_2 + ... & \rightarrow p_{1R}\ X_1 + p_{2R}\ X_2 ... \end{array} \tag{37}$$

The variation of a given compound X_i involved in R reactions is defined by:

$$\frac{dX_i}{dt} = \sum_{r=1}^{R} \eta_{ir} v_r = \eta_{i1} v_1 + \eta_{i2} v_2 + ... + \eta_{iR} v_R \tag{38}$$

where

v_r = rate of reaction r (with $r = 1, 2, ...R$):

$$v_r = k_r \prod_i X_i^{n_{ir}}$$

$\eta_{ir} = p_{ir} - n_{ir}$ = stoechiometric coefficient of compound X_i in reaction r.

We illustrate this on the following example:

r	reaction	rate	η_{Xr}	η_{Yr}
1	$A \xrightarrow{k_1} X$	$v_1 = k_1 A$	$\eta_{X1} = 1$	$\eta_{Y1} = 0$
2	$B + X \xrightarrow{k_2} Y + C$	$v_2 = k_2 BX$	$\eta_{X2} = -1$	$\eta_{Y2} = 1$
3	$2X + Y \xrightarrow{k_3} 3X$	$v_3 = k_3 X^2 Y$	$\eta_{X3} = 1$	$\eta_{Y3} = -1$
4	$X \xrightarrow{k_4} D$	$v_4 = k_4 X$	$\eta_{X4} = -1$	$\eta_{Y4} = 0$

The evolution equations for X and Y are given by (see eq. 38) :

$$\begin{cases} \dfrac{dX}{dt} = \eta_{X1} v_1 + \eta_{X2} v_2 + \eta_{X3} v_3 + \eta_{X4} v_4 \\ \dfrac{dY}{dt} = \eta_{Y1} v_1 + \eta_{Y2} v_2 + \eta_{Y3} v_3 + \eta_{Y4} v_4 \end{cases} \tag{39}$$

By substituting the values of η_{Xi}, η_{Yi} and v_i (see table here above), these equations become:

$$\begin{cases} \dfrac{dX}{dt} = k_1 A - k_2 BX + k_3 X^2 Y - k_4 X \\ \dfrac{dY}{dt} = k_2 BX - k_3 X^2 Y \end{cases} \tag{40}$$

1.4 Chemical equilibrium

Often, chemical reactions are not completely irreversible and the transformation of the products back to the substrates is possible. It is then more precise to write:

$$\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C} + \mathrm{D} \tag{41}$$

In general, the concentration of the various substrates and products tend to the equilibrium concentration characterized by the equilibrium constant:

$$K_{eq} = \frac{C_{eq} D_{eq}}{A_{eq} B_{eq}} \tag{42}$$

NB: It is not the case if one of the compound is volatile or forms a precipitate or is consumed in other chemical reaction (ex: metabolic pathways), or is extracted from the medium (ex: translocation in the nucleus of the cell).

When we write the evolution equation for a compound of such a reversible reaction, we get two terms, one for each reaction:

$$\frac{dA}{dt} = -k_1 AB + k_{-1} CD \tag{43}$$

At the equilibrium, we have:

$$\overrightarrow{v} = \overleftarrow{v} \tag{44}$$

$$k_1 A_{eq} B_{eq} = k_{-1} C_{eq} D_{eq} \tag{45}$$

$$\frac{k_1}{k_{-1}} = \frac{C_{eq} D_{eq}}{A_{eq} B_{eq}} = K_{eq} \tag{46}$$

Note: In biology we often need to describe the kinetics of complex formation (such as the dimerization of proteins or the binding of a substrate to an enzyme or a ligand to a receptor):

$$\mathrm{A} + \mathrm{B} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \mathrm{AB} \tag{47}$$

In this case, the equilibrium is determined by the dissociation constant $K_D = k_d/k_a$.

1.5 Effect of temperature - Arrhenius equation

Reaction rates generally depend on temperature. The Arrhenius equation gives the dependence of the rate constant k of a chemical reaction on the temperature (expressed in Kelvin):

$$k = Ae^{-E_A/RT} \tag{48}$$

where A is the pre-exponential factor, E_A is the activation energy, and R is the universal gas constant ($R = 8.31 Jmol^{-1}K^{-1}$).

Arrhenius plot

Taking the natural logarithm of Arrhenius'equation yields:

$$\ln k = \ln(A) - \frac{E_A}{R}\frac{1}{T} \tag{49}$$

Thus, when a reaction has a rate constant k that obeys Arrhenius'equation, a plot of $\ln(k)$ versus $1/T$ gives a straight line, whose gradient and intercept can be used to determine E_A and A.

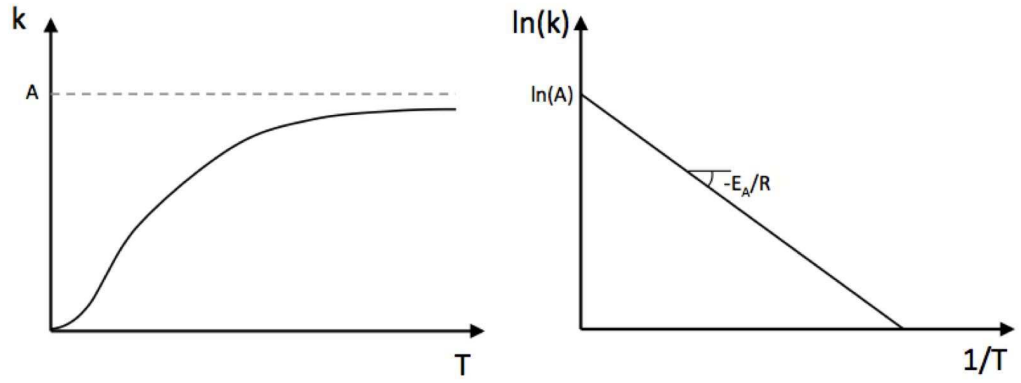


Figure 6: Arrhenius plot.

2 Enzyme kinetics

2.1 Enzymes

Enzymes are catalysts (generally proteins) that help to convert other molecules called substrates, into products, but they themselves are not changed by the reaction. Their most important features are catalytic power, specificity and regulation. Enzymes accelerate the conversion of substrates into products by lowering the free energy of activation of the reaction. For example, enzymes may aid in converting charge repulsions and allowing reacting molecules to come into contact for the formation of new chemical bounds. Or, if the reaction requires breaking of an existing bound, the enzyme may exert a stress on a substrate molecule, rendering a particular bound easily broken. Enzymes are particularly efficient at **speeding up** biological reactions, giving increase in speed up to 10^6 times or more. They are also **highly specific**, usually catalysing the reaction of only one particular substrate or closely related substrates. Finally, they are typically **regulated** by various positive and negative feedback systems, thus allowing precise control over the rate of reaction.

An example of enzymatic reaction is the first reaction of the glycolysis, catalysed by the enzyme hexokinase (Fig. 7):

$$\text{Glucose} + \text{ATP} \rightarrow \text{Glucose-6-phosphate} + \text{ADP} \tag{50}$$

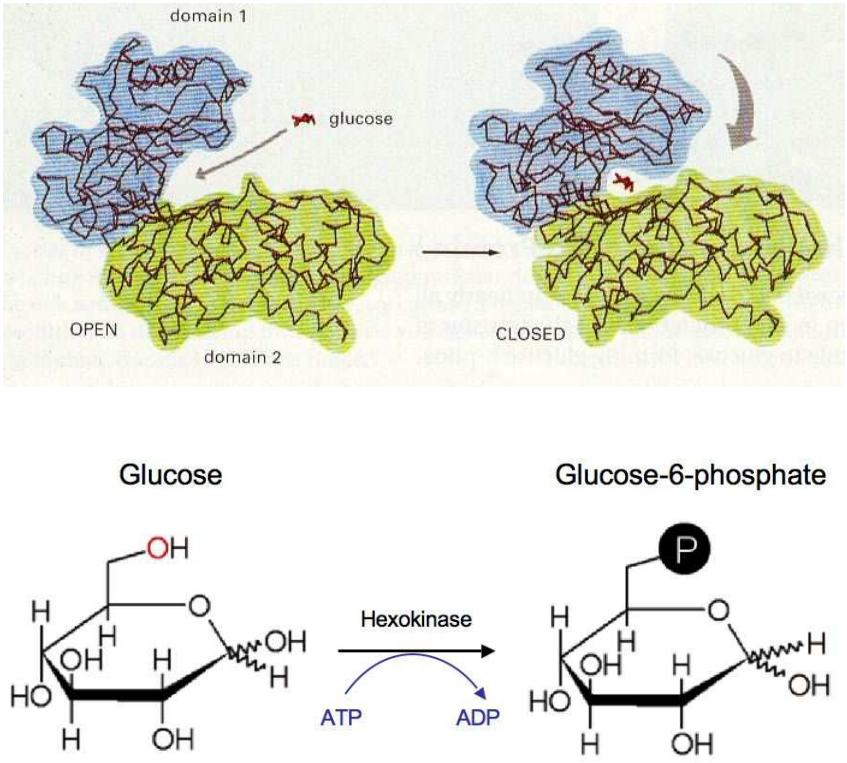


Figure 7: Hexokinase.

2.2 Mechanism of enzyme reactions

Enzymes accelerate reaction by stabilizing transition states of intermediary reactants, thereby lowering the activation energy required for the reaction (Fig. 8).

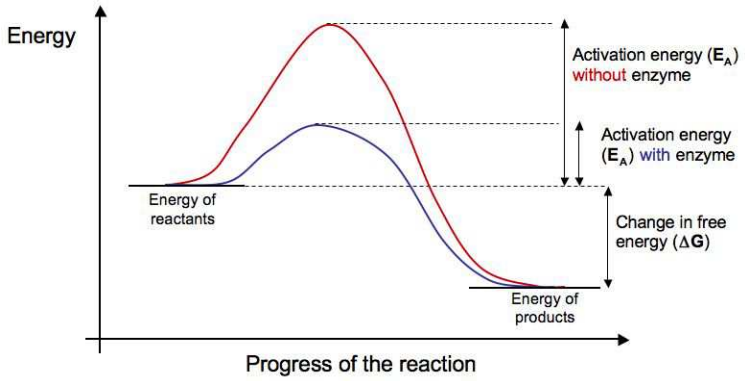


Figure 8: Activation Energy.

One of the first thing to realize about enzymes reaction is that they do not follow the law of mass action directly. As the concentration of substrate is increased, the rate of the reaction increases only to a certain extent, reaching a maximal reaction velocity at high substrate concentration. This is in contrast with the mass action law, which, when applied directly to the reaction with the enzyme predicts that the reaction velocity increases linearly as the substrate increases.

Figure 9: From left to right: Victor Henri (1872-1940) , Leonor Michaelis (1875-1949), Maud Menten (1879-1960), and Archibald Hill (1886-1977)

The work of Michaelis and Menten is based on the works of Victor Henri and of Adrian John Brown. They studied the mechanism of the enzyme invertase (=sucrase) which hydrolyzes sucrose into glucose and fructose and found that this reaction is initiated by a bond between the enzyme and the substrate. The study of other enzyme reactions led them to propose that the formation of enzyme-substrate complex is a general mechanism of enzyme reactions (Fig. 10). The activity of enzymes may also be regulated by co-factors, inhibitor, or activators (See Fig. 11 for an example of competitive inhibition).

We describe here the most common mechanisms to explain this saturation in speed (i.e. Michaelis-Menten and Briggs-Haldane equations), as well as the effect of inhibitors and activators on the kinetics. We will also discuss the Hill function, used to describe enzyme kinetics in presence of cooperativity, as well as the kinetics of allosteric enzymes.

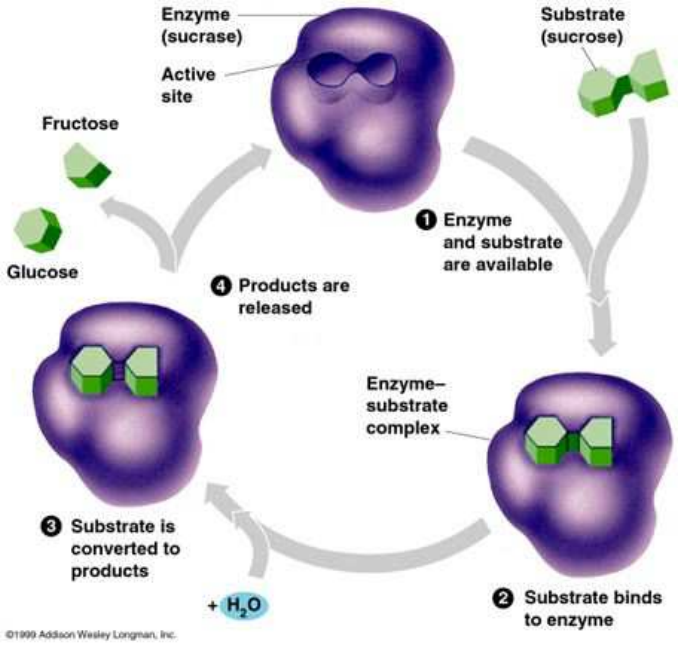


Figure 10: Mechanism of enzyme reactions.

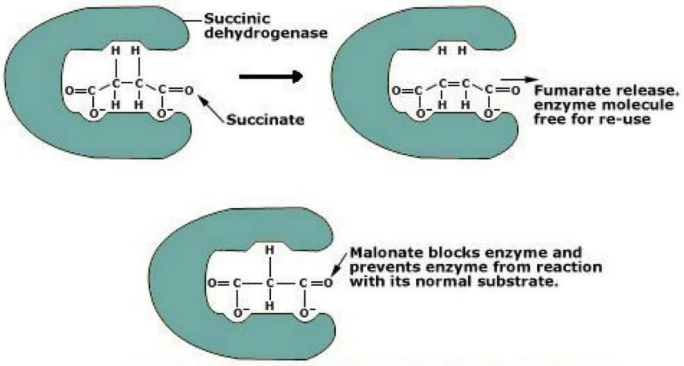


Figure 11: Example of competitive inhibition.

2.3 Equilibrium approximation: Michaelis-Menten equation

Based on experimental observations, Michaelis and Menten (1913) have proposed the following mechanism for the enzyme-catalysed biochemical reactions:

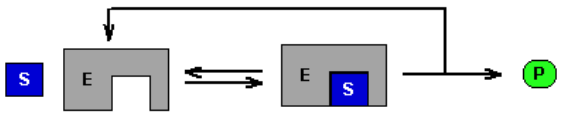


Figure 12: Michaelis-Menten mechanism.

The reaction scheme can be written (C=complex between E and S):

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \tag{51}$$

The evolution equations for the different species follow the mass action law:

$$\begin{cases} \dfrac{dS}{dt} = -k_1 ES + k_{-1} C \\ \dfrac{dE}{dt} = -k_1 ES + k_{-1} C + k_2 C \\ \dfrac{dC}{dt} = k_1 ES - k_{-1} C - k_2 C \\ \dfrac{dP}{dt} = k_2 C \end{cases} \tag{52}$$

In their original analysis, Michaelis and Menten assumed that the substrate S is in instantaneous equilibrium with the complex C, i.e.

$$k_1, k_{-1} >> k_2 \tag{53}$$

Thus

$$k_1 ES = k_{-1} C \tag{54}$$

Since $E_T = E + C$, we find that:

$$C = \frac{E_T S}{\dfrac{k_{-1}}{k_1} + S} \tag{55}$$

Hence, the product P of the reaction is produced at a rate

$$v = \frac{dP}{dt} = k_2 C = V_{max} \frac{S}{K_S + S} \tag{56}$$

where

$$V_{max} = k_2 E_T \text{ and } K_S = \frac{k_{-1}}{k_1}$$

2.4 Quasi-steady state assumption: Briggs-Haldane equation

Based on the same reaction mechanism (Fig. 12 and eqs. (52)), Briggs and Haldane (1925) suggested an alternative hypothesis: if the enzyme is present in "catalytic" amounts (i.e. $E \ll S$), then, very shortly after mixing E and S, a steady state is established in which the concentration of ES (variable C in eqs. 52) remains essentially constant with time (see Fig. 13):

$$\frac{dC}{dt} = \frac{dE}{dt} = 0 \tag{57}$$

We define E_{tot} the total concentration of enzyme: $E_{tot} = E + C =$ constant.

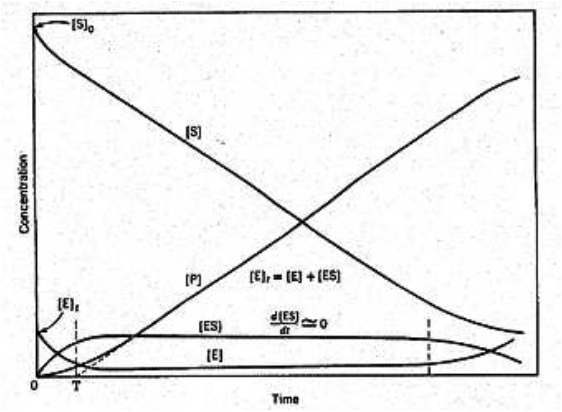


Figure 13: Evolution of the concentration in an enzyme-catalyzed reaction.

This hypothesis is the **quasi-steady state approximation** (see appendix for the detailed demonstration). This assumption implies that (see the second equation of eqs. (52) with the condition given by eq. (57)):

$$k_1 ES - k_{-1} C - k_2 C = 0 \tag{58}$$

From this equation, with $E_{tot} = E + C$, we can extract C:

$$C = \frac{k_1 E_{tot} S}{k_1 S + (k_{-1} + k_2)} = \frac{E_{tot} S}{S + \frac{(k_{-1}+k_2)}{k_1}} \tag{59}$$

When we replace this expression for C in the rate of appearance of P, we obtain:

$$v = \frac{dP}{dt} = k_2 C = \frac{k_2 E_{tot} S}{S + \frac{(k_{-1}+k_2)}{k_1}} \tag{60}$$

which is usually written as:

$$v = V_{max} \frac{S}{S + K_M} \tag{61}$$

where

$$K_M = \frac{(k_{-1} + k_2)}{k_1} \text{ and } V_{max} = k_2 E_{tot}$$

The rate is thus similar than in the case of the equilibrium hypothesis (Michaelis-Menten equation); only K_M has a slightly different meaning. We see that when $k_1, k_{-1} >> k_2$, we have $K_M \to K_S$. Note that K_M is usually called the *Michaelis-Menten* constant, although the exact meaning of this constant is rarely specified.

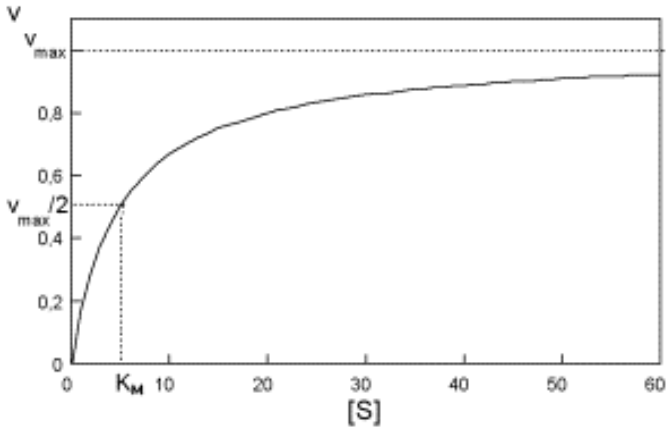


Figure 14: Michaelis-Menten kinetics.

Rewritten in the following manner, equation (61) gives a straight line, which is useful to determine the parameters K_M and V_{max} (Lineweaver-Burk representation):

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{S} \tag{62}$$

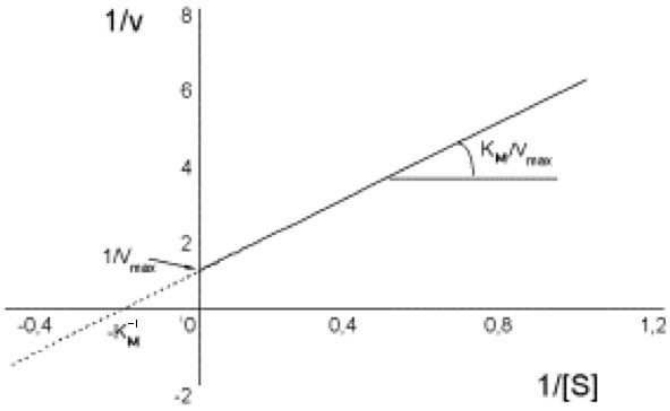


Figure 15: Michaelis-Menten kinetics (Lineweaver-Burk plot).

Examples of kinetic values are given in Appendix (see Table).

2.5 Reversible Michaelis-Menten kinetics

Many enzyme reactions are reversible and may be described by the following reaction scheme:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P} \tag{63}$$

Assuming that the total concentration of enzyme is constant,

$$E_T = E + ES \tag{64}$$

and using the quasi steady state approximation

$$\frac{dES}{dt} = 0 \tag{65}$$

we find that the product P of the reaction is produced at a rate

$$v = \frac{dP}{dt} = \frac{\frac{v_f S}{K_s} - \frac{v_b P}{K_p}}{1 + \frac{S}{K_s} + \frac{P}{K_p}} \tag{66}$$

where

$$v_f = k_2 E_T$$

$$v_b = k_{-1} E_T$$

are the maximum forward and backward reaction rates, and

$$K_s = \frac{k_{-1} + k_2}{k_1}$$

$$K_p = \frac{k_2 + k_{-1}}{k_{-2}}$$

At equilibrium, we have

$$v = \frac{dP}{dt} = 0 \tag{67}$$

i.e.

$$\frac{v_f S}{K_s} = \frac{v_b P}{K_p} \tag{68}$$

and

$$K_{eq} = \frac{P_{eq}}{S_{eq}} = \frac{v_f K_p}{v_b K_s} \tag{69}$$

This equation is known as the Haldane relationship.

2.6 Inhibition

Competitive inhibition

In the case of a competitive inhibition, the inhibitor is in competition with the substrate for the active site of the enzyme: either one or the other can bind the enzyme, but not both at the same time.

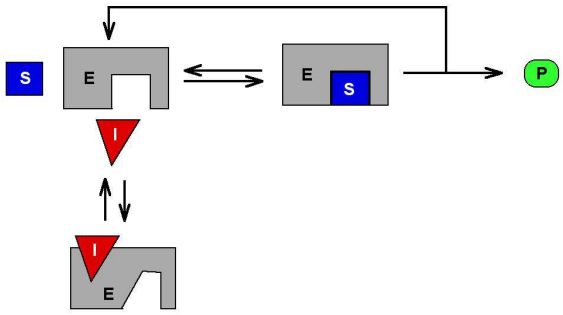


Figure 16: Competitive inhibition: mechanism.

The reaction scheme is:

$$\begin{aligned} &\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \\ &\mathrm{E} + \mathrm{I} \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} \mathrm{EI} \end{aligned} \tag{70}$$

The rate of appearance of P depends on the concentration of the inhibitor I in the following manner:

$$v = V_{max} \frac{S}{K_M \left(1 + \frac{I}{K_I}\right) + S} \tag{71}$$

where K_I is the equilibrium constant of the EI complex formation: $K_I = k_{-i}/k_i$.

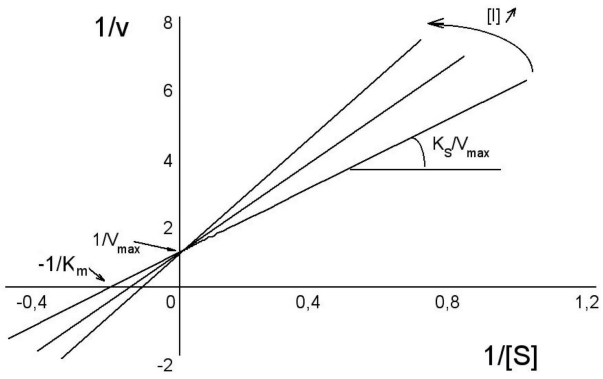


Figure 17: Competitive inhibition.

Uncompetitive inhibition

In the case of an uncompetitive (=anti-competitive) inhibition, the inhibitor is not in competition with the substrate for the active site of the enzyme. It binds only the substrate-enzyme complex. The substrate facilitates the binding of the inhibitor to the enzyme.

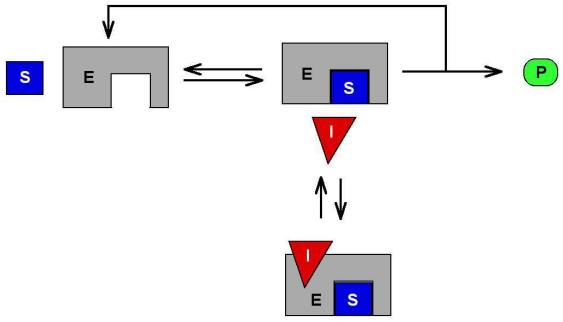


Figure 18: Anti-competitive inhibition: mechanism.

The reaction scheme is:

$$\begin{aligned} \mathrm{E} + \mathrm{S} &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \\ \mathrm{ES} + \mathrm{I} &\underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} \mathrm{ESI} \end{aligned} \tag{72}$$

The rate of appearance of P depends on the concentration of the inhibitor I in the following manner:

$$v = V_{max} \frac{\frac{S}{\left(1 + \frac{I}{K_I}\right)}}{\frac{K_M}{\left(1 + \frac{I}{K_I}\right)} + S} \tag{73}$$

where $K_I = k_{-i}/k_i$.

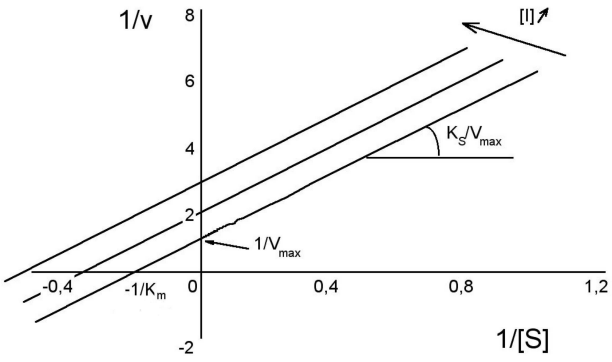


Figure 19: Anti-competitive inhibition.

Non-competitive inhibition

In the case of a non-competitive inhibition (also said mixed inhibition), both types of inhibition are present: the inhibitor can bind either the free enzyme or the enzyme-substrate complex.

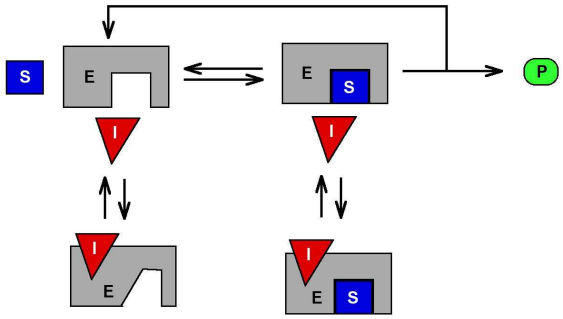


Figure 20: Non-competitive inhibition: mechanism.

The reaction scheme is:

$$\begin{aligned} \mathrm{E} + \mathrm{S} &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \\ \mathrm{E} + \mathrm{I} &\underset{k_{-i1}}{\overset{k_{i1}}{\rightleftharpoons}} \mathrm{EI} \\ \mathrm{ES} + \mathrm{I} &\underset{k_{-i2}}{\overset{k_{i2}}{\rightleftharpoons}} \mathrm{ESI} \end{aligned} \tag{74}$$

The rate of appearance of P depends on the concentration of the inhibitor I in the following manner:

$$v = \frac{V_{max}}{\left(1 + \frac{I}{K_{I1}}\right)} \frac{S}{\frac{K_M\left(1 + \frac{I}{K_{I1}}\right)}{\left(1 + \frac{I}{K_{I2}}\right)} + S} \tag{75}$$

where $K_{I1} = k_{-i1}/k_{i1}$ and $K_{I2} = k_{-i2}/k_{i2}$.

If $K_{I1} = K_{I2} = K_I$ (i.e. if the affinity of the inhibitor for the enzyme is independent on the binding of the substrate), eq. (75) can be reduced to:

$$v = \frac{V_{max}}{\left(1 + \frac{I}{K_I}\right)} \frac{S}{K_M + S} \tag{76}$$

2.7 Activation

Some enzymes need to be activated before to be bound to the substrate (case of essential activation).

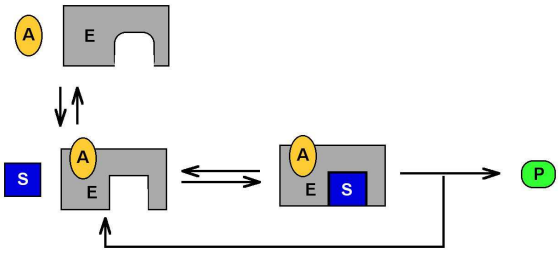


Figure 21: Activation: mechanism.

The reaction scheme is:

$$\begin{aligned} &\mathrm{E} + \mathrm{A} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \mathrm{EA} \\ &\mathrm{EA} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{EAS} \overset{k_2}{\rightarrow} \mathrm{EA} + \mathrm{P} \end{aligned} \tag{77}$$

The rate of appearance of P depends on the concentration of the activator A in the following manner:

$$v = \frac{V_{max} S}{K_M \left(1 + \frac{K_A}{A}\right) + S} \tag{78}$$

where $K_A = k_{-a}/k_a$.

Remark:

The scheme shown here is the case of an essential activation. If $A = 0$ (no activator), the reaction does not take place. There are also cases where the activator is not essential: the reaction occurs even in absence of the activator A, but at a lower speed. The derivation of the kinetic rate in that case in left as an exercise. In other cases, it is the substrate (and not the enzyme as considered here) that needs to be activated before being bound to the enzyme.

2.8 Two-substrate enzyme kinetics

In all the examples treated above, we considered reactions of a single substrate and a single product. Actually such reactions are rather rare in biochemistry. Strictly speaking, they are confined to isomerizations, such as the interconversion of glucose-1-phosphate and glucose-6-phosphate, catalyzed by phosphoglucomutase (Cornish-Bowden, 1995). Nevertheless, these developments of enzyme kinetics are used to describe and to model a large range of biochemical reactions. Many enzymes can be treated as single-substrate enzymes because the second substrate is usually present in large excess, so that its concentration can be treated as a constant (H_2O, NAD, ATP, etc). However, there is a number of cases where the two substrates are in comparable amount. For these cases, it is important to consider explicitely the binding of each substrate to the enzyme. Various mechanisms may be assumed. We present here the mechanism based on the formation of a ternay complex. Other mechanisms can be found in textbooks (e.g. Cornish-Bowden, 1995).

Consider the following reaction, catalyzed by enzyme E:

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{P} + \mathrm{Q} \tag{79}$$

A and B are two substrates. P and Q are the products. We assume that (1) A and B bind independently two different sites of the enzyme, (2) a ternary complex EAB is formed, and (3) once P and Q are formed they are released and the reverse reaction does not take place. This model is schematized in Fig. 22.

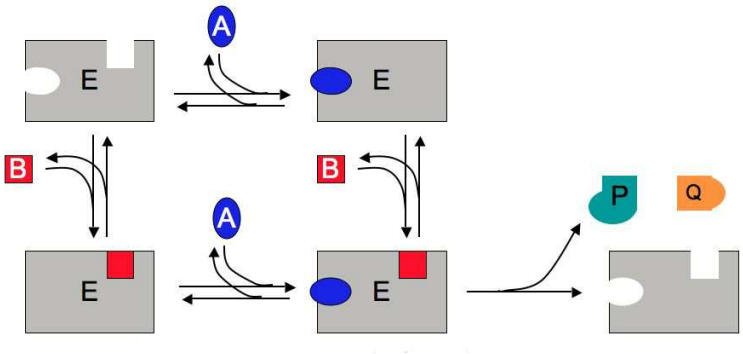
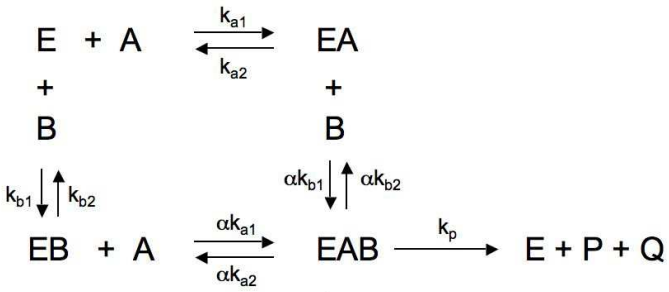


Figure 22: Two-substrate enzyme kinetics: mechanism with ternary complex (EAB).

The kinetic parameters are defined as follows:



The kinetic rates for the substrates A and B are given by:

$$\begin{aligned}\frac{dA}{dt} &= -k_{a1}.E.A + k_{a2}EA - \alpha k_{a1}.A.EB + \alpha k_{a2}.EAB \\ \frac{dB}{dt} &= -k_{b1}.E.B + k_{b2}EB - \alpha k_{b1}.B.EA + \alpha k_{b2}.EAB\end{aligned} \tag{80}$$

We assume that the total concentration of the enzyme is constant:

$$E_T = E + EA + EB + EAB = const \tag{81}$$

As previously, we will show that under the QSSA hypothesis, we can simplify the kinetic equations. The QSSA assumes that the (reversible) binding of A and B to the enzyme is fast compared to the conversion of A and B into the products and hence the binding/unbinding reactions can be set at the steady state:

$$\begin{aligned}k_{a1}.E.A &= k_{a2}EA \\ \alpha k_{a1}.A.EB &= \alpha k_{a2}.EAB \\ k_{b1}.E.B &= k_{b2}EB \\ \alpha k_{b1}.B.EA &= \alpha k_{b2}.EAB\end{aligned} \tag{82}$$

From Eqs. (82), we find:

$$\begin{aligned}EA &= \frac{k_{a1}}{k_{a2}}.E.A \\ EB &= \frac{k_{b1}}{k_{b2}}.E.B\end{aligned} \tag{83}$$

We can then replace EA and EB in Eq. (81):

$$E_T = E + \frac{k_{a1}}{k_{a2}}.E.A + \frac{k_{b1}}{k_{b2}}.E.B + EAB \tag{84}$$

and express EAB as a function of A and B:

$$\begin{aligned}EAB &= \frac{k_{a1}}{k_{a2}}\frac{k_{b1}}{k_{b2}}\left(\frac{E_T - EAB}{1 + \frac{k_{a1}}{k_{a2}}A + \frac{k_{b1}}{k_{b2}}B}\right)A.B \\ &= K_aK_b\left(\frac{E_T - EAB}{1 + K_aA + K_bB}\right)AB \\ &= \frac{\frac{K_a.K_b.E_T.A.B}{1 + K_a + K_bB}}{1 + \frac{K_aK_b.A.B}{1 + K_aAK_bB}} \\ &= \frac{K_aK_bBE_TAB}{1 + K_a.A + K_b.B + K_aK_b.A.B} \\ &= \frac{E_TAB}{\frac{1}{K_aK_b} + \frac{A}{K_b} + \frac{B}{K_a} + A.B}\end{aligned} \tag{85}$$

The rate of production of the product P and Q is thus given by:

$$v = \frac{dP}{dt} = k_p.EAB = k_p \frac{E_T AB}{\frac{1}{K_a K_b} + \frac{A}{K_b} + \frac{B}{K_a} + A.B} \tag{86}$$

or, defining v_{max} as $k_p E_T$

$$v = v_{max} \frac{AB}{\frac{1}{K_a K_b} + \frac{A}{K_b} + \frac{B}{K_a} + A.B} \tag{87}$$

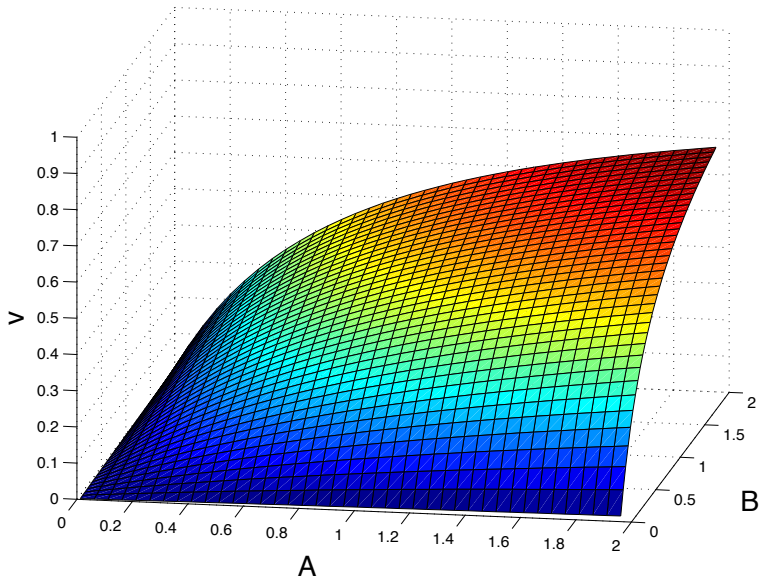


Figure 23: Two substrates kinetics

Note that if B is constant, the equation becomes

$$v = v'_{max} \frac{A}{K' + A} \tag{88}$$

where

$$v'_{max} = \frac{v_{max} B}{\frac{1}{K_b} + B} \text{ and } K' = \frac{\frac{1}{K_a K_b} + \frac{B}{K_a}}{\frac{1}{K_b} + B} \tag{89}$$

2.9 Substrate competition

Different substrates may compete for the same enzyme. For irreversible reactions, substrate competition is comparable to enzyme inhibition (see above). We consider here the case of competition between 2 substrates (S_1 and S_2) for the same enzyme (E) with reversible reactions (Schauble et al, 2013).

The reaction scheme is:

$$\begin{array}{lll} \mathrm{E} + \mathrm{S}_1 & \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} & \mathrm{ES}_1 \quad \underset{k_{-21}}{\overset{k_{21}}{\rightleftharpoons}} \quad \mathrm{E} + \mathrm{P}_1 \\ \mathrm{E} + \mathrm{S}_2 & \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} & \mathrm{ES}_2 \quad \underset{k_{-22}}{\overset{k_{22}}{\rightleftharpoons}} \quad \mathrm{E} + \mathrm{P}_2 \end{array} \tag{90}$$

The kinetics rates are:

$$\frac{dS_1}{dt} = -k_{11}E.S_1 + k_{-11}ES_1 \tag{91}$$

$$\frac{dS_2}{dt} = -k_{12}E.S_2 + k_{-12}ES_2 \tag{92}$$

$$\begin{aligned} \frac{dE}{dt} &= -k_{11}E.S_1 + k_{-11}ES_1 + k_{21}ES_1 - k_{-21}E.P_1 \\ &\quad -k_{12}E.S_2 + k_{-12}ES_2 + k_{22}ES_2 - k_{-22}E.P_2 \end{aligned} \tag{93}$$

$$\frac{dES_1}{dt} = k_{11}E.S_1 - k_{-11}ES_1 - k_{21}ES_1 + k_{-21}E.P_1 \tag{94}$$

$$\frac{dES_2}{dt} = k_{12}E.S_2 - k_{-12}ES_2 - k_{22}ES_2 + k_{-22}E.P_2 \tag{95}$$

Making the QSSA assumption

$$\frac{dES_1}{dt} = \frac{dES_2}{dt} = 0$$

we find:

$$ES_2 = ES_1 \frac{(k_{-11} + k_{21})(k_{12}S_2 + k_{-22}P_2)}{(k_{11}S_1 + k_{-21}P_1)(k_{-12} + k_{22})} \tag{96}$$

Since the total amount of enzyme is constant ($E_T = E + ES_1 + ES_2$), we have

$$ES_2 = E_T - ES_1 - E \tag{97}$$

$$ES_1 \frac{(k_{-11} + k_{21})(k_{12}S_2 + k_{-22}P_2)}{(k_{11}S_1 + k_{-21}P_1)(k_{-12} + k_{22})} = E_T - ES1 - \frac{(k_{-11} + k_{21})ES_1}{k_{11}S_1 + k_{-21}P_1} \tag{98}$$

$$E = \frac{E_T}{1 + \dfrac{k_{11}S_1 + k_{-21}P_1}{k_{-11} + k_{21}} + \dfrac{k_{12}S_2 + k_{-22}P_2}{k_{-12} + k_{22}}} \tag{99}$$

The rate of appearance of P_1 is then:

$$\frac{dP_1}{dt} = k_{21}ES_1 - k_{-21}E.P_1 \tag{100}$$

$$= \frac{k_{21}E_T\frac{S_1}{K_{M11}} - k_{-11}E_T\frac{P_1}{K_{M21}}}{\frac{S_1}{K_{M11}} + \frac{P_1}{K_{M21}} + \frac{S_2}{K_{M12}} + \frac{P_2}{K_{M22}} + 1} \tag{101}$$

where

$$K_{M11} = \frac{k_{11}}{k_{-11} + k_{21}} \tag{102}$$

$$K_{M21} = \frac{k_{-21}}{k_{-11} + k_{21}} \tag{103}$$

$$K_{M12} = \frac{k_{12}}{k_{-12} + k_{22}} \tag{104}$$

$$K_{M22} = \frac{k_{-22}}{k_{-12} + k_{22}} \tag{105}$$

Similarly, the rate of appearance of P_2 is

$$\frac{dP_2}{dt} = k_{22}ES_2 - k_{-22}E.P_2 \tag{106}$$

$$= \frac{k_{22}E_T\frac{S_2}{K_{M12}} - k_{-11}E_T\frac{P_2}{K_{M22}}}{\frac{S_1}{K_{M11}} + \frac{P_1}{K_{M21}} + \frac{S_2}{K_{M12}} + \frac{P_2}{K_{M22}} + 1} \tag{107}$$

2.10 Cooperativity: Hill function

Some enzymes have several active sites. The binding of a molecule of substrate to one site may or not influence the binding of another molecule of substrate to the second site. The two sites are independent in the first case, while they are dependent (cooperative) in the second case. We discuss here both cases. Then we generalised to the case of an enzyme having n cooperative binding sites.

Two independent active sites

We first discuss the case of an enzyme with two independent binding sites.

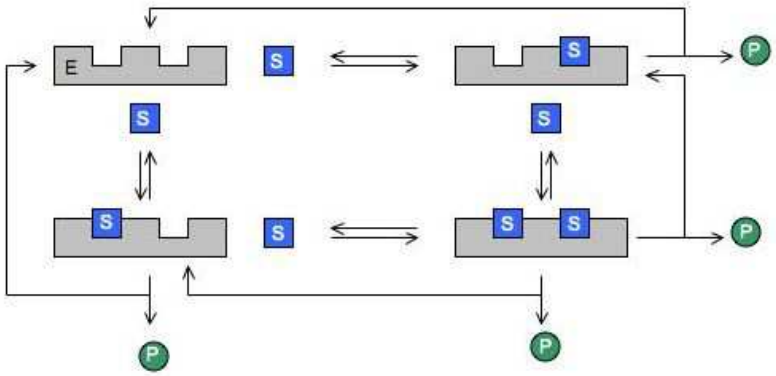


Figure 24: Enzyme with two binding sites: mechanism.

The reaction scheme is as follows:

$$\begin{aligned} 2 \times \left[\mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C}_1 \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \right] \\ 2 \times \left[\mathrm{S} + \mathrm{C}_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{C}_2 \overset{k_4}{\rightarrow} \mathrm{C}_1 + \mathrm{P} \right] \end{aligned} \tag{108}$$

We define

$$E_T = E + 2C_1 + C_2 \tag{109}$$

The rate of apparition of P is given by:

$$v = 2k_2C_1 + 2k_4C_2 \tag{110}$$

NB: In the rhs, the first "2" stands because there are 2 forms of C_1, while the second "2" stands for the fact that there are 2 catalytic sites on C_2.

The evolution equations are:

$$\begin{cases} \dfrac{dS}{dt} = 2(-k_1SE + k_{-1}C_1 - k_3SC_1 + k_{-3}C_2) \\ \dfrac{dC_1}{dt} = 2(k_1SE - (k_{-1} + k_2)C_1 - k_3SC_1 + (k_{-3} + k_4)C_2) \\ \dfrac{dC_2}{dt} = 2(k_3SC_1 - (k_{-3} + k_4)C_2) \end{cases} \tag{111}$$

The quasi steady state approximation allows:

$$\frac{dC_1}{dt} = \frac{dC_2}{dt} = 0 \tag{112}$$

Defining

$$K_1 = \frac{k_{-1} + k_2}{k_1} \text{ and } K_2 = \frac{k_{-3} + k_4}{k_3} \tag{113}$$

we find:

$$C_1 = \frac{SE}{K_1} \text{ and } C_2 = \frac{SC_1}{K_2} = \frac{S^2E}{K_1K_2} \tag{114}$$

The two binding sites are assumed to be independent. This means that

$$\begin{aligned} k_1 &= k_3 = k_+ \\ k_{-1} &= k_{-3} = k_- \\ k_2 &= k_4 = k_p \end{aligned} \tag{115}$$

Combining eq. (110) and (109), with (115), we have

$$\frac{v}{E_T} = 2\frac{k_pC_1 + k_pC_2}{E + 2C_1 + C_2} \tag{116}$$

Replacing C_1 and C_2 by their expressions (eqs. 114), we get

$$\frac{v}{E_T} = \frac{2\left(\frac{SE}{K_1} + \frac{S^2E}{K_1K_2}\right)}{E + 2\frac{SE}{K_1} + \frac{S^2E}{K_1K_2}} \tag{117}$$

Noting

$$K = K_1 = K_2 = \frac{k_- + k_p}{k_+} \tag{118}$$

we find

$$\begin{aligned} v &= 2k_pE_T\frac{(K+S)S}{K^2 + 2KS + S^2} \\ v &= 2k_pE_T\frac{(K+S)S}{(K+S)^2} \\ v &= 2k_pE_T\frac{S}{(K+S)} \end{aligned} \tag{119}$$

Therefore,

$$v = V_{max}\frac{S}{(K+S)} \tag{120}$$

where

$$V_{max} = 2k_pE_T \text{ and } K = \frac{k_- + k_p}{k_+}$$

The rate has a similar form as in the case of Michaelis-Menten. The maximum rate is simply two times the rate of a one binding site enzyme.

Two cooperative active sites

The binding of the susbtrate can sometimes be cooperative, which means that the binding of one molecule of substrate favors the binding of other molecules of substrate to the neighbour binding sites.

This is the case if, in the reaction scheme (108),

$$k_3 >> k_1 \tag{121}$$

Then we have

$$K_2 = \alpha K_1 \tag{122}$$

with

$$\alpha << 1$$

and

$$C_1 = \frac{SE}{K_1} << C_2 = \frac{1}{\alpha}\frac{S^2E}{K_1^2} \tag{123}$$

Thus

$$v = \frac{2k_pE_T\left(\frac{S}{K_1} + \frac{S^2}{\alpha K_1^2}\right)}{1 + 2\frac{S_1}{K_1} + \frac{S^2}{\alpha K_1^2}} \tag{124}$$

For $S \simeq K_1$ we find

$$\begin{aligned} v &\simeq \frac{V_{max}\frac{S^2}{\alpha K_1^2}}{1 + \frac{S^2}{\alpha K_1^2}} \\ v &\simeq \frac{V_{max}S^2}{K + S^2} \end{aligned} \tag{125}$$

where

$$K = \alpha K_1^2 \tag{126}$$

We see here that in the case of cooperative binding sites, the rate does not follow a Michaelian function anymore. This function, called Hill function, has a sigmoidal shape.

$$v = V_{max}\frac{S^2}{K + S^2} \tag{127}$$

where

$$K = \alpha K_1^2$$

Generalisation: n cooperative active sites

The reaction scheme for an enzyme with 4 binding sites can be represented as follows, where K_i denotes the equilibrium (dissociation) constant of the ith step of binding: $K_i = \overleftarrow{k}_i / \overrightarrow{k}_i$. Cooperativity implies that $K_1 > K_2 > K_3 > K_4$. In other words, the more S molecules are already bound, the easier the binding of additionnal S molecules becomes.

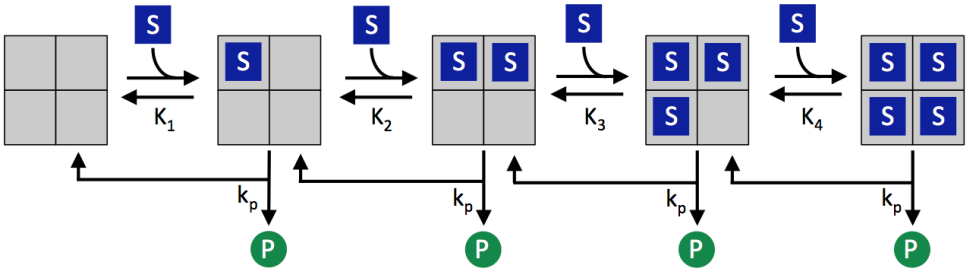


Figure 25: Cooperativity: mechanism.

If we assume that the binding of substrate is cooperative and that all forms of the enzyme-substrate complex (ES1, ES2, ES3 and ES4) are able to transform S into P, the rate of apparition of P is:

$$v = V_{max} \frac{S^n}{K^n + S^n} \tag{128}$$

where V_{max} is function of k_P and E_{tot} (with $E_{tot} = E + ES1 + ES2 + ES3 + ES4$):

$$V_{max} = n k_p E_{tot}$$

and K is function of the K_i. If $K_i = \alpha_i K_{i-1}$,

$$K^n = K_1^n \prod_{i=1}^{n} \alpha_i^{n-i} = K_1^n (\alpha_1^{n-1} \alpha_2^{n-2} ...) \tag{129}$$

The curve defined by eq. (128) has a sigmoidal shape, with $v = V_{max}/2$ at $S = K$.

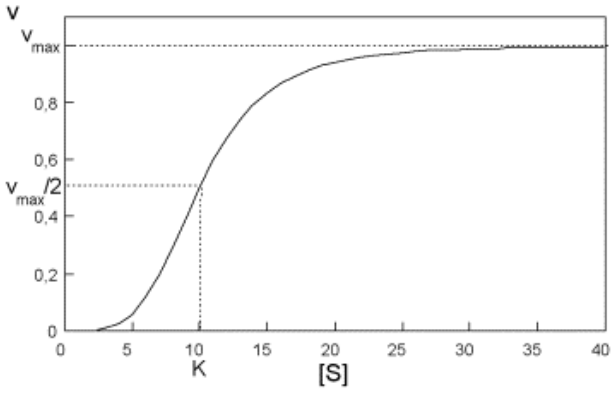


Figure 26: Hill kinetics.

Remark: It is important to stress that the Hill coefficient n is not equal to the number of binding sites. In fact, n tends to the number of binding sites when the cooperativity is very strong. In practice, however, the cooperativity is never infinite and n is generally less than the number of binding sites (and can take non-integer values).

Equation (27) can be transformed to show a linear relation, as in the Lineweaver-Burk representation of Michaelis-Menten equation:

$$\frac{v}{V_{max}} = \frac{S^n}{K^n + S^n} \tag{130}$$

$$S^n \frac{V_{max} - v}{v} = K^n \tag{131}$$

$$\log\left(\frac{v}{V_{max} - v}\right) = n \log S - n \log K \tag{132}$$

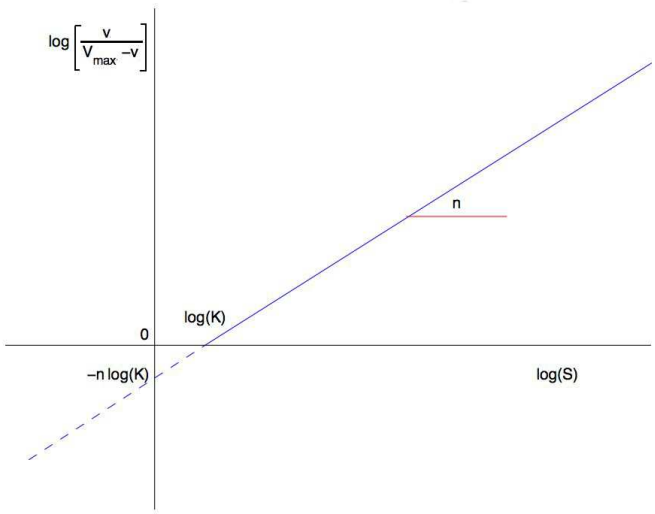


Figure 27: Hill kinetics.

2.11 Allosteric model

Monod, Changeux and Jacob (1963) studied many examples of cooperative and allosteric phenomena, and concluded that they were closely related and that conformational flexibility probably accounted for both. Subsequently Monod, Wyman and Changeux (1965) proposed a general model to explain both phenomena within a simple set of postulates. The model is often referred to as the allosteric model.

The allosteric model starts from the observation that each molecule of a typical cooperative protein contains several subunits. We will denote by n the number of subunits (Fig. 28A).

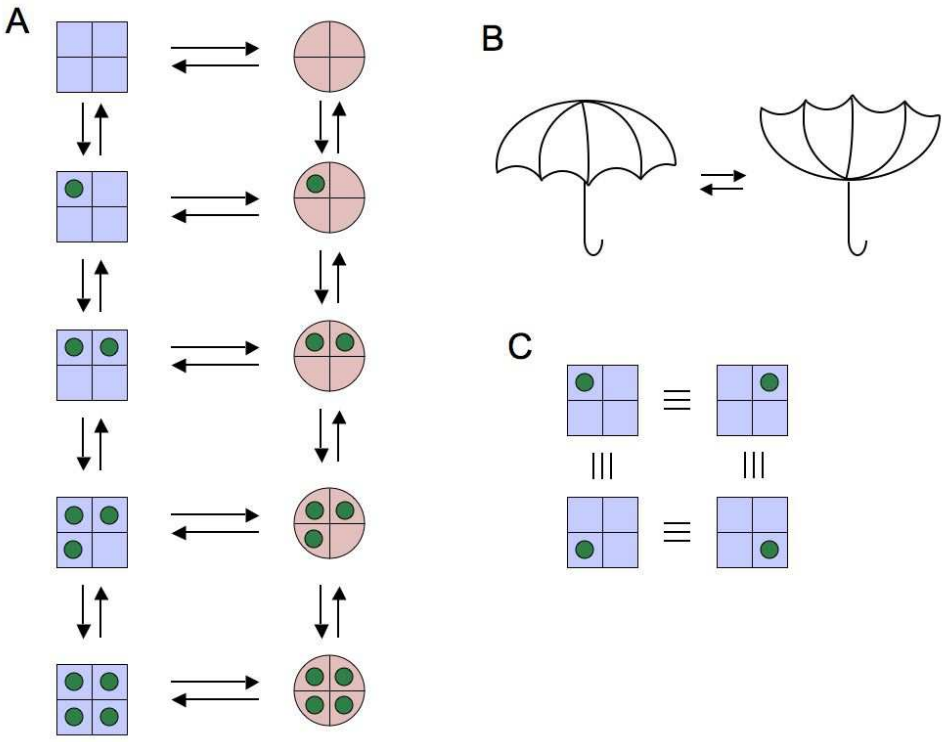


Figure 28: Allosteric model.

The model then relies on the following assumptions:

- Each subunit can exist in two different conformations, designed R and T. These labels originally stood for *relaxed* and *tense*, from the idea that the protein had to relax in order to bind substrate.
- All subunits of the enzyme must be in the same conformation at any time (*umbrella effect*, Fig. 28B). Hence, for a dimeric protein the conformational states R_2 and T_2 are the only ones permitted, the mixed conformation RT being forbidden (this condition becomes much more restrictive when the enzyme counts more than 2 subunits (e.g. for $n = 4$ the allowed states are R_4 and T_4, while R_3T, R_2T_2, RT_3 are all forbidden).

- The two states of the protein are in equilibrium, with an equilibrium (allosteric) constant L=[R_2]/[T_2].
- A ligand (substrate) A can bind to a subunit in either conformation, but the dissociation constant are different: $K_R = [R][A]/[RA]$ for each R subunit; $K_T = [T][A]/[TA]$ for each T subunit. The ratio $c = K_R/K_T < 1$. In other words the affinity of the substrate is not the same for the two forms.

We describe here the derivation of the equations for the case of an enzyme with 2 subunits. We then discuss the generalization to the case of n subunits.

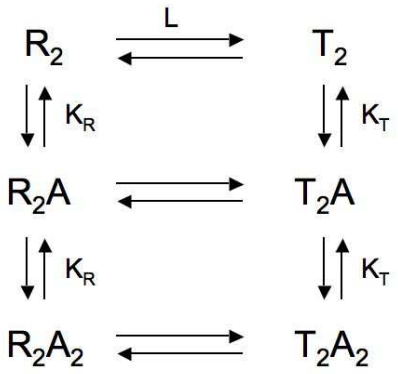


Figure 29: Scheme of the allosteric model.

The assumptions listed above imply the set of equilibria between the various states shown in Fig. 29 ($R_2 \rightleftharpoons T_2$, R_2+A $\rightleftharpoons$ R_2A, R_2A + A $\rightleftharpoons$ R_2A_2, etc.) and the concentrations of the 6 forms of the protein are related by the following expressions:

$$\begin{aligned}
[R_2A] &= 2[R_2][A]/K_R \\
[R_2A_2] &= \frac{1}{2}[R_2A][A]/K_R = [R_2][A]^2/K_R^2 \\
[T_2] &= L[R_2] \\
[T_2A] &= 2[T_2][A]/K_T = 2L[R_2][A]/K_T \\
[T_2A_2] &= \frac{1}{2}[T_2A][A]/K_T = L[R_2][A]^2/K_T^2
\end{aligned} \tag{133}$$

In each equation the factor 2, 1/2 or 1 results from the fact that the dissociation constants are defined in terms of individual sites but the expression are written for the complete molecules. For example $K_R = [R][A]/[RA] = 2[R_2][A]/[R_2A]$, because there are two vacant sites in each R_2 molecule and one occupied site in each R_2A molecule (see also Fig. 28C).

The fractional saturation Φ is defined as the fraction of sites occupied by the ligand:

$$\begin{aligned}
\Phi &= \frac{\text{number of sites occupied by the ligand}}{\text{total number of sites}} \\
&= \frac{[R_2A] + 2[R_2A_2] + [T_2A] + 2[T_2A_2]}{2([R_2] + [R_2A] + [R_2A_2] + [T_2] + [T_2A] + [T_2A_2])}
\end{aligned} \tag{134}$$

In the numerator the concentration of each molecule is counted according to the number of occupied sites is contains (the empty sites are not counted), but in the denominator, each molecule is counted according to how many sites it contains, whether it is occupied or not.

Substituing the concentrations from Eqs. (133) into Eq. (134), we get:

$$\begin{aligned}\Phi &= \frac{[A]/K_R + [A]^2/K_R^2 + L[A]/K_T + L[A]^2/K_T^2}{1 + 2[A]/K_R + [A]^2/K_R^2 + L + 2L[A]/K_T + L[A]^2/K_T^2} \\ &= \frac{(1+[A]/K_R)[A]/K_R + L(1+[A]/K_T)[A]/K_T}{(1+[A]/K_R)^2 + L(1+[A]/K_T)^2}\end{aligned} \tag{135}$$

For the general case where the enzyme has n subunits, Eq. (135) becomes:

$$\Phi = \frac{(1+[A]/K_R)^{n-1}[A]/K_R + L(1+[A]/K_T)^{n-1}[A]/K_T}{(1+[A]/K_R)^n + L(1+[A]/K_T)^n} \tag{136}$$

The shape of the saturation curve defined by Eqs (136) depends on the values of n, L, and K_R/K_T, as can be illustrated by assiging some extreme values to these constants.

If $n = 1$, i.e. if there is only one binding site per molecule, the equation simplifies to

$$\Phi = \frac{[A]}{K_{RT} + [A]} \text{ where } K_{RT} = \frac{1+L}{1/K_R + L/K_T} \tag{137}$$

is the dissociation constant that takes account for the fact that both R and T forms participate in the binding. The complexity of this dissociation constant does not however alter the fact that it is a constant, and thus no cooperativity is possible if $n = 1$.

If $L = 0$, the T form of the protein does not exist under any condition, and the factor $(1 + [A]/K_R)^{n-1}$ cancels between the numerator and the denominator, leaving

$$\Phi = \frac{[A]}{K_R + [A]} \tag{138}$$

which predicts hyperbolic (non-cooperative) binding with dissociation constant K_R. A similar simplification occurs if L approaches infinity, i.e. if the R form does not exist. In this case, $\Phi = [A]/(K_T + [A])$. It follows that both R and T forms are needed if cooperativity is to be possible.

It is also necessay for the two forms to be functionally different from each other, i.e. $K_R \neq K_T$. If $K_R = K_T$ it is again possible to cancel the common factor $(1 + [A]/K_R)^{n-1}$, leaving an hyperbolic expression. This illustrates the reasonable expectation that if the ligand binds equally well to the two states of the enzyme, the relative proportion in which they exist are irrelvant to the binding behaviour.

If $K_T >> K_R$, i.e. if A binds only to the R state, we find:

$$\Phi = \frac{(1+[A]/K_R)[A]/K_R}{L + (1+[A]/K_R)^2} \tag{139}$$

When [A] is sufficiently large, then L at the denominator becomes negligeable and the curve approaches a hyperbola. But when $[A]$ is small, the constant L dominates the

denominator and causes Φ to rise very slowly from the origin as $[A]$ increases from zero. In other words, as long as L is significantly different from zero the curve of Φ against $[A]$ must be sigmoidal.

The curve Φ, as defined by Eq. (136) is plotted in Fig. 30 for various parameter values.

If we assume that A is a substrate of the allosteric enzyme, which transforms A into a product P, then the kinetics rate v of appearance of P can write:

$$v = \frac{d[P]}{dt} = v_{max}\Phi \tag{140}$$

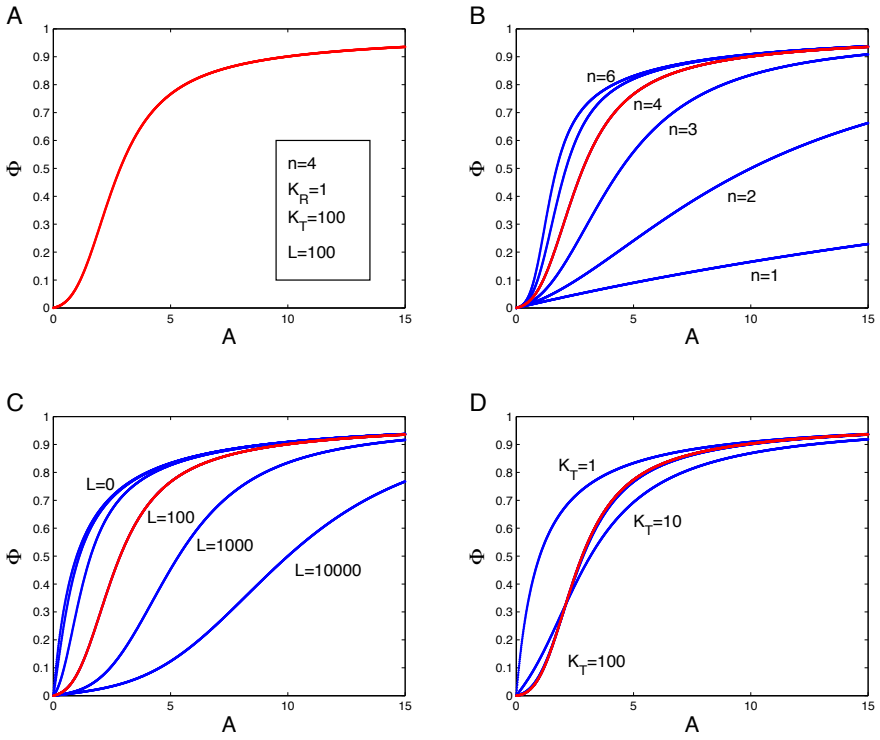


Figure 30: Plot of Φ as a function of $[A]$ for various sets of parameter values. (A) Default parameter values ($n = 4$, $K_R >> K_T, L >> 1$. (B) Effect of the number of subunits, n. (C) Effect of the allosteric constant, L. (D) Effect of the affinity ratio c (controlled by changing K_T, K_R being fixed).

2.12 Zero-order ultrasensitivity

Goldbeter and Koshland showed how ultrasensitivity may arise in a system based on the covalent modification of a protein. They consider a protein that can exist in two forms, e.g. a phosphorylated, active form (W^*) and a unphosphorylated, inactive form (W), and that the conversion is catalyzed by two different enzymes (e.g. a kinase E_1 and a phosphatase E_2). The scheme of such a system is depicted in Fig. 31.

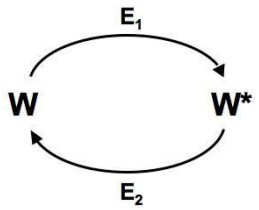


Figure 31: Scheme

Assuming a molecular mechanism similar to the one used to derive the Michaelis-Menten equation, the detailed reaction scheme is as follows:

$$\begin{array}{l} \mathrm{W} + \mathrm{E}_1 \underset{d_1}{\overset{a_1}{\rightleftharpoons}} \mathrm{WE}_1 \overset{k_1}{\rightarrow} \mathrm{W}^* + \mathrm{E}_1 \\ \mathrm{W}^* + \mathrm{E}_2 \underset{d_2}{\overset{a_2}{\rightleftharpoons}} \mathrm{W}^*\mathrm{E}_2 \overset{k_2}{\rightarrow} \mathrm{W} + \mathrm{E}_2 \end{array} \tag{141}$$

The corresponding evolution equations are:

$$\begin{array}{l} \frac{d[W]}{dt} = -a_1[W][E_1] + d_1[WE_1] + k_2[W^*E_2] \\ \frac{d[WE_1]}{dt} = a_1[W][E_1] - (d_1 + k_1)[WE_1] \\ \frac{d[W^*]}{dt} = -a_2[W^*][E_2] + d_2[W^*E_2] + k_1[WE_1] \\ \frac{d[W^*E_2]}{dt} = a_2[W^*][E_2] - (d_2 + k_2)[W^*E_2] \end{array} \tag{142}$$

We assume that the total concentration of W, E_1, and E_2 are constant:

$$\begin{array}{l} W_T = [W] + [W^*] + [WE_1] + [W^*E_2] \\ E_{1T} = [E_1] + [WE_1] \\ E_{2T} = [E_2] + [W^*E_2] \end{array} \tag{143}$$

The steady state can be obtained by solving:

$$\begin{array}{l} a_1[W][E_1] - d_1[E_1] = k_1[WE_1] = k_2[W^*E_2] \\ a_2[W^*E_2] - d_2[E_2] = k_1[WE_1] = k_2[W^*E_2] \end{array} \tag{144}$$

Thus, at steady state:

phosphorylation rate = dephosphorylation rate

$$k_1[WE_1] = k_2[W^*E_2] \tag{145}$$

We define the fraction of the active and inactive forms of the protein at steady state:

$$\begin{aligned} W^* &= \frac{[W^*]}{W_T} \\ W &= \frac{[W]}{W_T} \end{aligned} \tag{146}$$

Suppose

$$[WE_1], [W^*E_2] << [W], [W^*] \tag{147}$$

when

$$W_T >> E_{1T}, E_{2T} \tag{148}$$

Then

$$W_T \approx [W] + [W^*] \tag{149}$$

$$\begin{aligned} &k_1[WE_1] = a_1[W][E_1] - d_1[WE_1] \\ &(k_1 + d_1)[WE_1] = a_1[W](E_{1T} - [WE_1]) \\ &\left(\frac{k_1 + d_1}{a_1}\right)[WE_1] = [W]E_{1T} - [W][WE_1] \end{aligned} \tag{150}$$

Thus,

$$[WE_1] = \frac{[W]E_{1T}}{K_{m1} + [W]} \tag{151}$$

with

$$K_{m1} = \left(\frac{k_1 + d_1}{a_1}\right) \tag{152}$$

Similarly, we find:

$$[W^*E_1] = \frac{[W^*]E_{2T}}{K_{m2} + [W^*]} \tag{153}$$

with

$$K_{m2} = \left(\frac{k_2 + d_2}{a_2}\right) \tag{154}$$

We define the maximum rates of E_1 and E_2:

$$\begin{aligned} v_1 &= k_1 E_{1T} \\ v_2 &= k_2 E_{2T} \end{aligned} \tag{155}$$

Relation (145) thus writes

$$\begin{aligned} &k_1[WE_1] = k_2[W^*E_2] \\ &k_1 \frac{[W]E_{1T}}{K_{m1} + [W]} = k_2 \frac{[W^*]E_{2T}}{K_{m2} + [W^*]} \\ &v_1 \frac{[W]}{K_{m1} + [W]} = v_2 \frac{[W^*]}{K_{m2} + [W^*]} \end{aligned} \tag{156}$$

Defining the molar fractions

$$\begin{aligned} w^* &= \frac{[W^*]}{[W_T]} \\ w &= \frac{[W]}{[W_T]} \\ w + w^* &= 1 \end{aligned} \tag{157}$$

and the normalized Michaelian constants:

$$\begin{aligned} K_1 &= \frac{K_{m1}}{[W_T]} \\ K_2 &= \frac{K_{m2}}{[W_T]} \end{aligned} \tag{158}$$

we obtain

$$\frac{v_1(1-w^*)}{K_1 + (1-w^*)} = v_2 \frac{w^*}{K_2 + w^*} \tag{159}$$

or, after rearranging the equation:

$$\frac{v_1}{v_2} = \frac{w^*(K_1 + 1 + w^*)}{(1-w^*)(K_2 + w^*)} \tag{160}$$

w^* is solution of a second-degree equation:

$$w^* \left(\frac{v_1}{v_2} - 1\right) - w^* \left[\left(\frac{v_1}{v_2} - 1\right) - K_2 \left(\frac{v_1}{v_2} + \frac{K_1}{K_2}\right)\right] - K_2 \left(\frac{v_1}{v_2}\right) \tag{161}$$

Let's call

$$\phi = \left(\frac{v_1}{v_2} - 1\right) - K_2 \left(\frac{v_1}{v_2} + \frac{K_1}{K_2}\right) \tag{162}$$

Then

$$w^* = \frac{\phi + \left[\phi^2 + 4\left(\frac{v_1}{v_2} - 1\right) K_2 \left(\frac{v_1}{v_2}\right)\right]^{1/2}}{2\left(\frac{v_1}{v_2} - 1\right)} \tag{163}$$

In the particular case where $v_1 = v_2$, we find

$$\begin{aligned} v_2(1-w^*)(K_2 + w^*) &= v_2 w^*(K_1 + 1 - w^*) \\ w^* &= \frac{K_2}{K_1 + K_2} \\ w^* &= \frac{1}{1 + \frac{K_1}{K_2}} \end{aligned} \tag{164}$$

More generally, $v_1 \neq v_2$, so how does vary w^* with v_1/v_2?

Let's first look at the case $K_1, K_2 >> 1$. In that case, Eq. (159) becomes

$$\frac{v_1 w}{K_1} = \frac{v_2 w^*}{K_2} \tag{165}$$

i.e.

$$w^* = \frac{\frac{v_1}{v_2}}{\frac{K_1}{K_2} + \frac{v_1}{v_2}} \tag{166}$$

In the case where $K_1, K_2 << 1$, the curve for $w*$ (defined by Eq. (163)) takes the form of a sigmoid with a very sharp threshold (ultra-sensitivity) (Fig. 32).

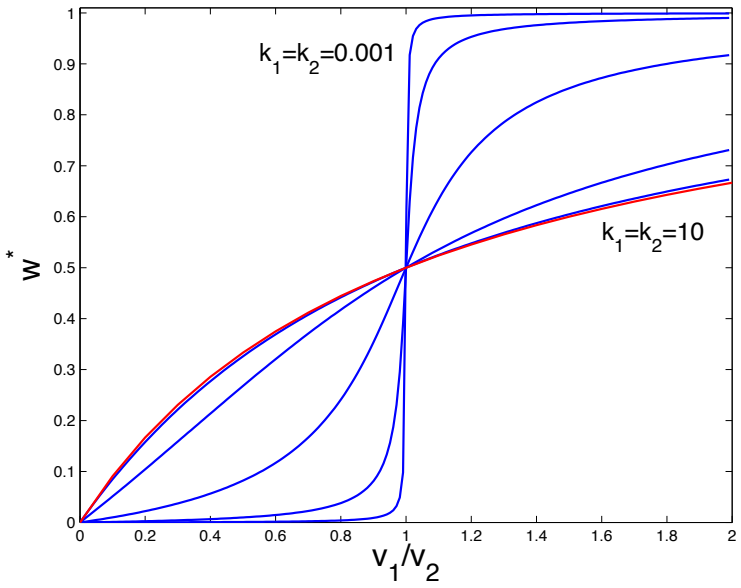


Figure 32: Fraction of active (phosphorylated) protein as a function of the ratio v_1/v_2. The red curve correspond to the approximation (166) and the blue curves correspond to Eq. (163), for various values of $K_1 = K_2$.

3 Gene regulation

3.1 Transcription, regulation, and transcription factors

Transcription of a gene is the process by which RNA polymerase produces mRNA (messenger RNA) that corresponds to the gene coding sequence. The mRNA is then translated into a protein, the gene product. The rate at which the gene is transcribed, i.e. the number of mRNA molecules produced per unit time, is controlled by the promoter, a regulatory region of DNA that very often precedes the gene. RNA polymerase binds a specific binding site (DNA sequence) at the promoter, thereby leading to the assembly of a multimolecular transcription machinery.

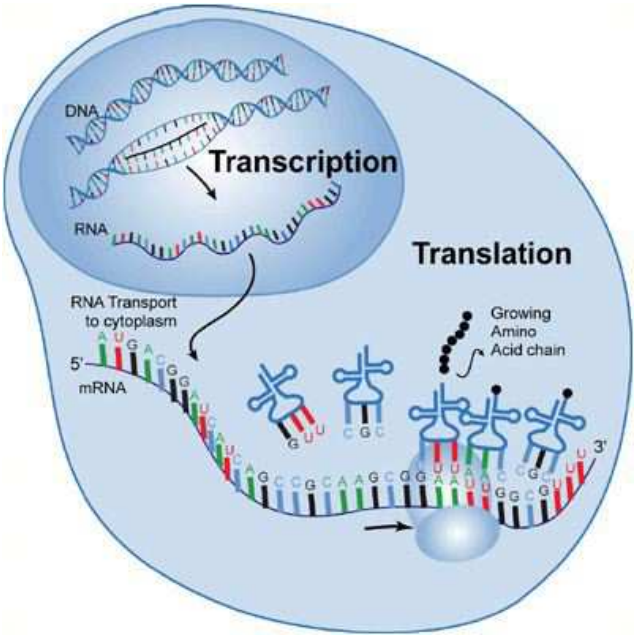


Figure 33: Transcription - Translation

Whereas RNA polymerase acts on virtually all of the genes, the expression of specific genes is very often regulated by proteins called transcription factors. These transcription factors affect the transcription rate by binding to specific sites in the promoter of the genes. When bound they change the probability per unit time that RNA polymerase binds the promoter and produces an mRNA molecule. Transcription factors can act as activators that increase the transcription rate of a gene, or as repressors that reduce the transcription rate.

In some cases, an activator may even be required for the transcription to occur (case of "essential" activators). The activity of these regulators can also be controlled by complex formation with small molecules (e.g. the inducer of repressor lacI in the case of the *lac* operon of *E. coli* or by formation of homomeric or heteromeric complexes. Competition between activators and inhibitors for a given binding site can also occur, and be crucial for an appropriate gene regulation. Finally, the situation is even more complex if, in a promoter of a given gene, multiple binding sites are present, being specific for one or

several regulators, and possibly leading to cooperative binding.

Transcription factors are proteins that are themselves encoded by genes, which possibly are regulated by other transcription factors, which in turn are regulated by other transcription factors, and so on. Such a set of interactions forms a transcriptional network.

In this section, we have selected a few regulatory mechanisms to illustrate how the kinetics of gene regulation can be derived. These schemes are very simplified and, of course, numerous variants and more detailed models can be elaborated.

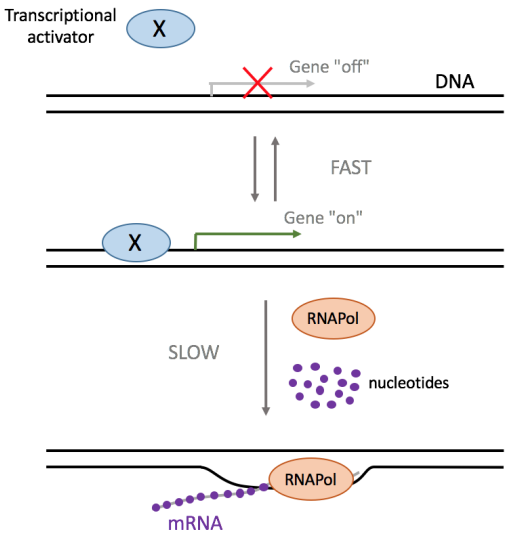


Figure 34: Transcription: 2-state model for gene regulation: The gene must be activated (turned "on") through the binding of a transcription factor to be transcribed.

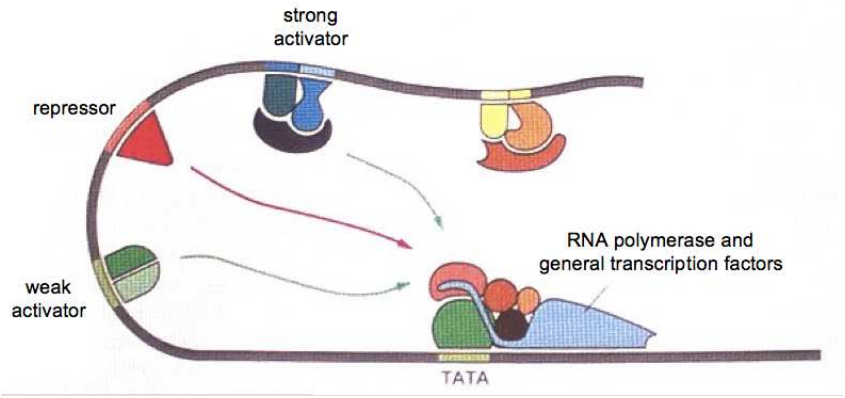


Figure 35: Regulation of gene expression by multiple transcription factors

3.2 Transcriptional activation

A regulator (protein X) is synthesized at a rate k_s and degraded (or consumed in another reaction) at a rate k_d. This regulator can reversibly bind the binding site D of the gene Y (denoted D_0 if unbound and D_1 if bound). The binding/unbinding rates are denoted by k_1 and k_{-1}. Only when activated by the regulator X, the transcription of gene Y can start (fig. 36). The transcription is ensured by the RNA polymerase, P, and requires a set of nucleotides $\{y_i\}$. In a second step, Y mRNA is translated into Y protein. The (lumped) transcription/translation rate is noted k_t.

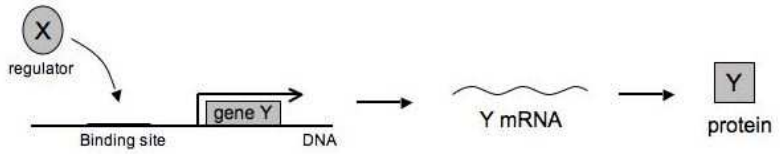


Figure 36: Transcriptional activation: A single regulator (X) is required to activate the transcription of a gene (Y). We also assume that the promoter contains a single binding site for protein X.

The reaction scheme assumed for this case is the following:

$$\begin{array}{c} \xrightarrow{k_s} \mathrm{X} \xrightarrow{k_d} \\ \mathrm{X} + \mathrm{D}_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{D}_1 \\ \mathrm{D}_1 + \ \mathrm{P} + \{y_i\} \xrightarrow{k_t} \mathrm{D}_1 + \mathrm{P} + \mathrm{Y} \end{array} \tag{167}$$

In this scheme, we can distinguish several time scales (fast vs slow reactions): The binding/unbinding of the regulatory protein to DNA can occur several times by second, while processes like protein synthesis and gene transcription last over several minutes. The protein and mRNA degradation rates are more variable; the life time of these compounds can range from a few seconds to several days.

To simplify, we have condensed the transcription of gene Y and the translation of Y mRNA into a single step.

The kinetics of the above reaction scheme can be written as :

$$\begin{aligned} \frac{dX}{dt} &= k_s - k_1 D_0 X + k_{-1} D_1 - k_d X \\ \frac{dD_1}{dt} &= k_1 D_0 X - k_{-1} D_1 \\ \frac{dY}{dt} &= k_t P Q D_1 \end{aligned} \tag{168}$$

where $\mathrm{Q} = \prod_i y_i =$ constant (we assume that the number of available nucleotides are not limiting).

Because of the fast binding-unbinding rate (k_1 and k_{-1} high), we can apply the quasi-steady state assumption for the binding/unbinding of the regulator X :

$$\frac{dD_0}{dt} = \frac{dD_1}{dt} = 0 \tag{169}$$

This leads to:

$$k_1 D_0 X = k_{-1} D_1 \tag{170}$$

Defining $D_T = D_0 + D_1$ the total number of genes or plasmids per unit volume (total concentration of binding sites), we find:

$$k_1 D_T X = (k_1 X + k_{-1}) D_1 \tag{171}$$

$$D_1 = \frac{k_1 D_T X}{k_{-1} + k_1 X} = \frac{D_T X}{K_1 + X} \tag{172}$$

where K_1 is the dissociation constant

$$K_1 = \frac{k_{-1}}{k_1} \tag{173}$$

The larger the dissociation constant, the higher the rate of dissociation of complex D_1, that is the weaker the binding of X to the promoter.

We find

$$\frac{dY}{dt} = k_t P Q \frac{D_T X}{K_1 + X} = v_s \frac{D_T X}{K_1 + X} \tag{174}$$

where $v_s = k_t P Q D_T =$ constant.

We can also note that the quasi-steady state assumption leads to:

$$\frac{dX}{dt} = k_s - k_d X \tag{175}$$

and thus the steady state of X depends only on its synthesis and degradation rates:

$$X_s = k_s / k_d \tag{176}$$

At steady state, we thus have

$$\frac{dY}{dt} = v_s \frac{D_T X_s}{K_1 + X_s} \tag{177}$$

Remark: Many DNA-transcription factors complex dissociate within less than 1 second, (i.e. $k_{-1} > 1 s^{-1}$). Therefore, we can average over times much longer than 1 sec and show, in particular for a single binding site (which is either free or occupied), that D_1/D_T is the probability that a site D is bound, averaged over many binding and unbinding events. When site D is bound, RNA polymerase can bind the promoter and transcribe the gene (Fig. 37).

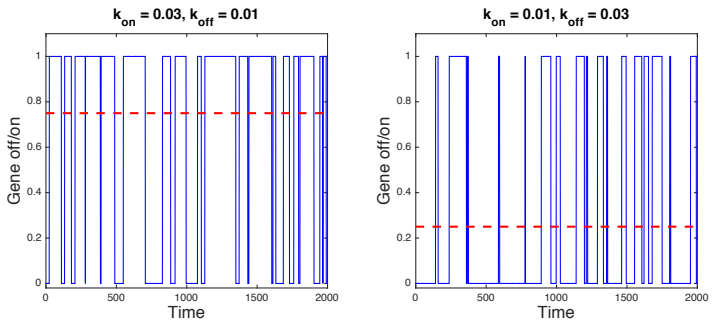


Figure 37: Activation (binding) / Deactivation (dissociation) dynamics of the gene.

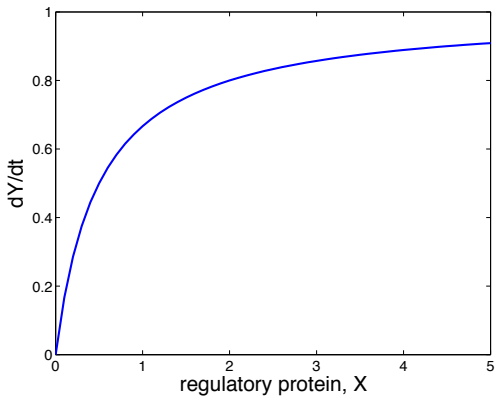


Figure 38: Kinetics of transcriptional activation. The prodution of Y as a function of the regulatory protein X follows a hyperbolic curve (eq. 174).

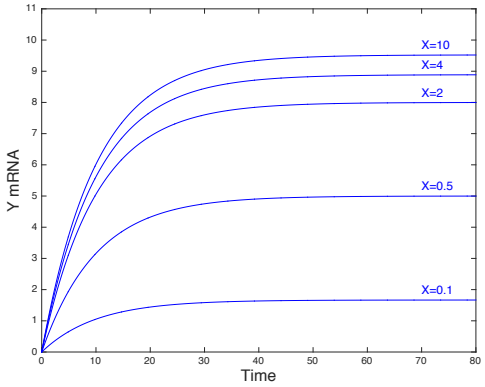


Figure 39: Time evolution of mRNA level for different concentrations of transcription factor X. To allow mRNA to reach a steady state, we assume that it is degraded (1st-order kinetics): $\frac{dY}{dt} = v_s \frac{D_T X_s}{K + X_s} - k_m Y$, with $v_s = 1$, $K = 0.5$, $D_T = 1$, and $k_m = 0.1$.

3.3 Transcriptional activation with auto-regulation

In this second study case, we assume that the regulatory protein X regulates the transcription of its own gene, X. In addition, we assume that both D_0 and D_1 can lead to the transcription of the gene X, but with different efficiency (fig. 40).

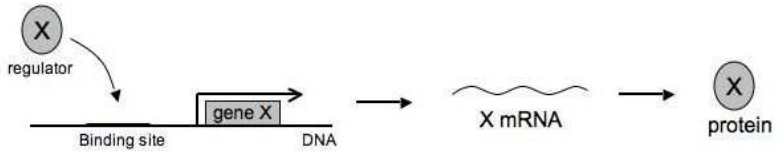


Figure 40: Transcriptional activation with auto-regulation: A regulatory protein X activates the transcription of its own gene.

The reaction scheme is as followed:

$$\begin{aligned} \mathrm{X} &\xrightarrow{k_d} \\ \mathrm{X} + \mathrm{D}_0 &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{D}_1 \\ \mathrm{D}_0 + \ \mathrm{P} + \{x_i\} &\xrightarrow{k_t} \mathrm{D}_0 + \mathrm{P} + \mathrm{X} \\ \mathrm{D}_1 + \ \mathrm{P} + \{x_i\} &\xrightarrow{\alpha k_t} \mathrm{D}_1 + \mathrm{P} + \mathrm{X} \end{aligned} \tag{178}$$

The corresponding kinetics equations are written:

$$\begin{aligned} \frac{dX}{dt} &= k_t PQD_0 + \alpha k_t PQD_1 - k_1 D_0 X + k_{-1} D_1 - k_d X \\ \frac{dD_0}{dt} &= -k_1 X D_0 + k_{-1} D_1 \\ \frac{dD_1}{dt} &= k_1 X D_0 - k_{-1} D_1 = -\frac{D_0}{dt} \end{aligned} \tag{179}$$

The quasi-steady state assumption, $\frac{dD_0}{dt} = \frac{dD_1}{dt} = 0$, leads to:

$$k_1 X D_0 = k_{-1} D_1 \tag{180}$$

With the definitions $K_1 = \frac{k_{-1}}{k_1}$ and $D_T = D_0 + D_1$ we find:

$$D_1 = \frac{D_T X}{K_1 + X} \tag{181}$$

and the evolution of X becomes:

$$\frac{dX}{dt} = k_t PQ(D_T - D_1) + \alpha k_t PQD_1 - k_d X \tag{182}$$

$$\begin{aligned}\frac{dX}{dt} &= k_t P Q D_T \left(1 + \frac{(\alpha - 1)X}{K_1 + X}\right) - k_d X \\ &= v_s \left(1 + \frac{(\alpha - 1)X}{K_1 + X}\right) - k_d X \end{aligned} \tag{183}$$

Depending on the value of α, the auto-regulation of X leads to either an activation or a repression of its own gene:

$\alpha = 1$	constitutive expression
$\alpha > 1$	auto-activation
$\alpha < 1$	auto-inhibition

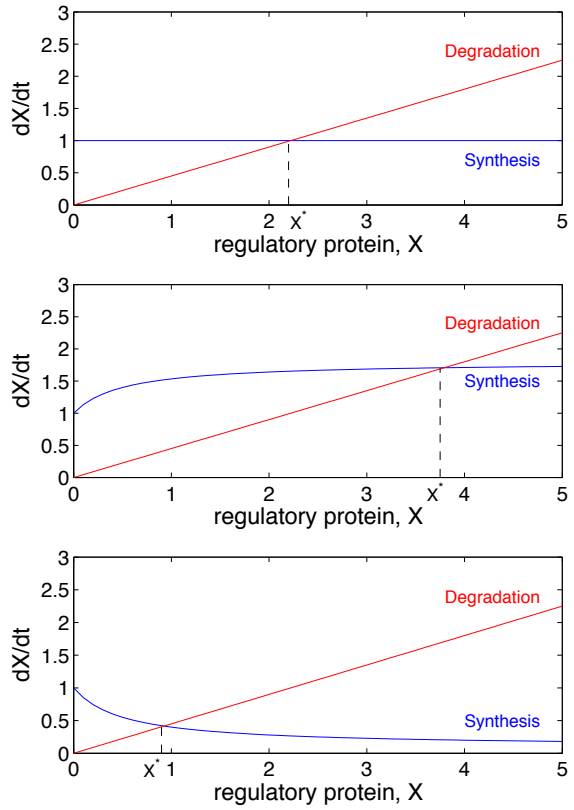


Figure 41: Auto-regulation of the gene X. Upper panel: $\alpha = 1$ (constitutive expression). Middel panel: $\alpha > 1$ (activation). Bottom panel: $\alpha < 1$ (repression).

3.4 Transcriptional activation with multiple binding sites

Here, we assume that there are two binding sites in the promoter of the gene Y and that the regulatory protein can bind these two binding sites, with a different affinity (Fig. 42).

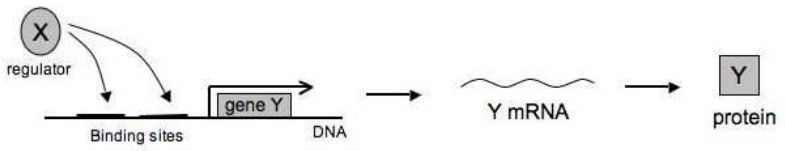


Figure 42: Transcriptional activation with multiple binding sites: A single regulatory protein X binds two binding sites to activate the repression of a gene Y.

The reaction scheme is as followed:

$$\begin{aligned} &\xrightarrow{k_s} \mathrm{X} \xrightarrow{k_d} \\ \mathrm{X} + \mathrm{D}_0 &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{D}_1 \\ \mathrm{X} + \mathrm{D}_1 &\underset{k_{-1}}{\overset{\alpha k_1}{\rightleftharpoons}} \mathrm{D}_2 \\ \mathrm{D}_2 + P + \{y_i\} &\xrightarrow{k_t} \mathrm{D}_2 + P + Y \end{aligned} \tag{184}$$

The corresponding kinetics equations are written:

$$\begin{aligned} \frac{dX}{dt} &= k_s - k_1 D_0 X + k_{-1} D_1 - \alpha k_1 D_1 X + k_{-1} D_2 - k_d X \\ \frac{dD_0}{dt} &= -k_1 D_0 X + k_{-1} D_1 \\ \frac{dD_1}{dt} &= k_1 D_0 X - k_{-1} D_1 - \alpha k_1 D_1 X + k_{-1} D_2 \\ \frac{dD_2}{dt} &= \alpha k_1 D_1 X - k_{-1} D_2 \end{aligned} \tag{185}$$

With the quasi-steady state assumption, $\frac{dD_0}{dt} = \frac{dD_1}{dt} = \frac{dD_2}{dt} = 0$, we have:

$$\alpha k_1 D_1 X = k_{-1} D_2 \tag{186}$$

With the definition $K_1 = k_{-1}/k_1$, we find

$$D_2 = \frac{\alpha}{K_1} D_1 X \text{ and } D_1 = \frac{D_0 X}{K_1} \tag{187}$$

$$D_2 = \frac{\alpha D_0 X^2}{K_1^2} \tag{188}$$

Defining D_T as previously, $D_T = D_0 + 2D_1 + D_2$, we get:

$$D_0 = \frac{D_T}{1 + 2\dfrac{X}{K_1} + \dfrac{\alpha X^2}{K_1^2}} \tag{189}$$

If we assume that the gene is transcribed only if the two binding sites are occupied, the evolution of protein Y is equal to:

$$\frac{dY}{dt} = k_t P Q D_2 = v_s \frac{\alpha X^2/K_1^2}{1 + 2X/K_1 + \alpha X^2/K_1^2} \tag{190}$$

where $v_s = k_t P Q D_T$ is a constant.

Two situations can be distinguished: either the two binding sites are independent or they are cooperative. If the binding sites are independent and identical, then $\alpha = 1$ and the above equation can be simplified as:

$$\frac{dY}{dt} = v_s \left(\frac{X/K_1}{1 + X/K_1} \right)^2 \tag{191}$$

If we assume cooperativity between the binding sites, then $\alpha >> 1$ and we get

$$\frac{dY}{dt} = v_s \left(\frac{\alpha X^2/K_1^2}{1 + 2X/K_1 + \alpha X^2/K_1^2} \right) \tag{192}$$

which can be approximated by:

$$\frac{dY}{dt} \approx v_s \frac{\alpha (X/K_1)^2}{1 + \alpha (X/K_1)^2} \tag{193}$$

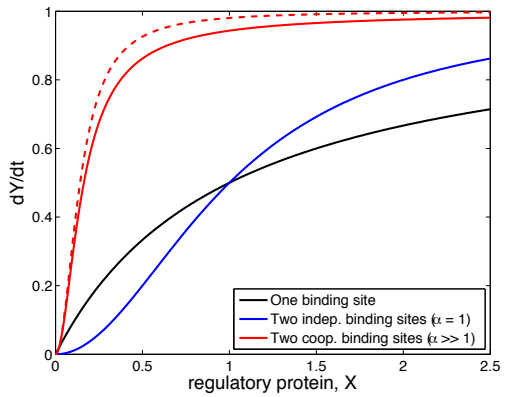


Figure 43: Multiple binding sites. Comparison of the dynamics in the case of a single binding site (eq. (174), black curve), independent (eq. (191), blue curve), and cooperative binding sites (eq. (192), red solid curve, or eq. (193), red dashed curve).

Generalization

For n binding sites, and in presence of cooperativity, the rate takes the form:

$$\frac{dY}{dt} = v_s \frac{\alpha(X/K_1)^n}{1 + \alpha(X/K_1)^n} \tag{194}$$

which can be rewritten as:

$$\frac{dY}{dt} = v_s \frac{X^n}{K^n + X^n} \tag{195}$$

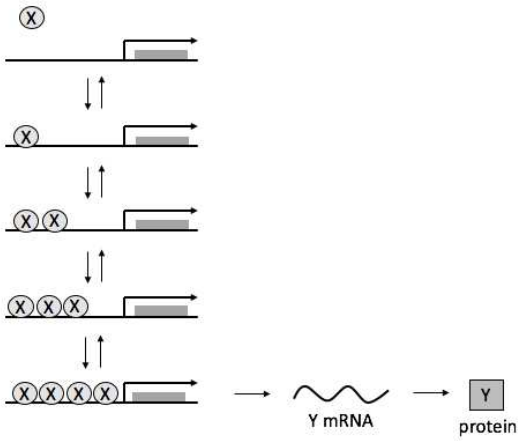


Figure 44: Scheme of gene activation when there are multiple (possibly cooperative) binding sites. Note that when cooperativity is high, the intermediate states remain at a low level and including a possible transcription of these states does not change much the kinetics.

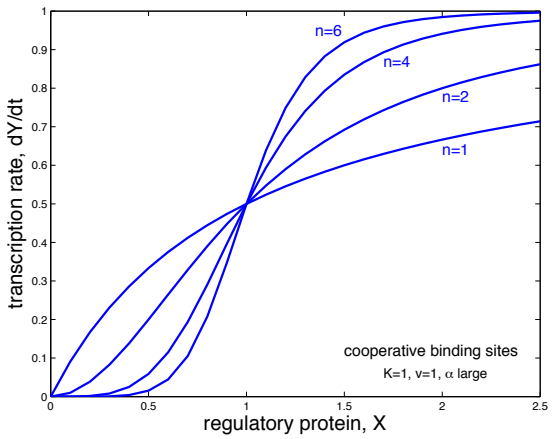


Figure 45: Multiple binding sites. Effect of the number of binding sites, n. High cooperativity. See Eq. (195).

3.5 Transcriptional activation by a dimeric complex

Here, we assume that the regulatory protein X must form a homodimer X_2 before binding the regulatory site.

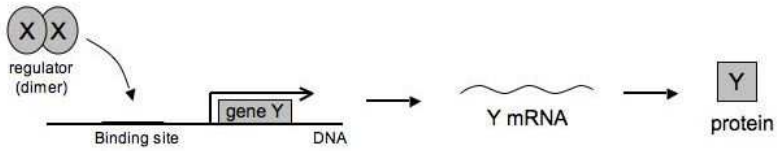


Figure 46: Transcriptional activation by a dimeric complex: The regulatory protein X forms a dimer than can bind the binding site and activate the transcription of gene Y.

The reaction scheme is as followed:

$$\begin{aligned} &\xrightarrow{k_s} \mathrm{X} \xrightarrow{k_d} \\ \mathrm{X} + \mathrm{X} &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{X}_2 \\ \mathrm{X}_2 + \mathrm{D}_0 &\underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{D}_1 \\ \mathrm{D}_1 + \mathrm{P} + \{y_i\} &\xrightarrow{k_t} \mathrm{D}_1\mathrm{+P+Y} \end{aligned} \tag{196}$$

The corresponding kinetics equations are written:

$$\begin{aligned} \frac{dX}{dt} &= k_s - k_d X - 2k_1 X^2 + 2k_{-1} X_2 \\ \frac{dX_2}{dt} &= k_1 X^2 - k_{-1} X_2 - k_2 D_0 X_2 + k_{-2} D_1 \\ \frac{dD_1}{dt} &= k_2 D_0 X_2 - k_{-2} D_1 \end{aligned} \tag{197}$$

With the quasi-steady state hypothesis $\frac{dD_1}{dt} = 0$ and the definition $D_T = D_0 + D_1$, we find

$$D_1 = \frac{D_T X_2}{K_2 + X_2} \tag{198}$$

If, in addition, we assume that the dimerisation rate is also fast (k_2 and k_{-2} high), we can do the hypothesis that

$$\frac{dX_2}{dt} = 0 \tag{199}$$

Then X_2 is given by

$$X_2 = \frac{X^2}{K_1} \tag{200}$$

and

$$D_1 = \frac{D_T X^2/K_1}{K_2 + X^2/K_1} = \frac{D_T X^2}{K_1 K_2 + X^2} \tag{201}$$

and the evolution of Y becomes:

$$\frac{dY}{dt} = k_t PQD_1 = v_s \frac{X^2}{K_1 K_2 + X^2} \tag{202}$$

where $v_s = k_t PQD_T$ is a constant.

Equation (202) has a sigmoidal form, similar to the case of two cooperative binding sites (eq. 192).

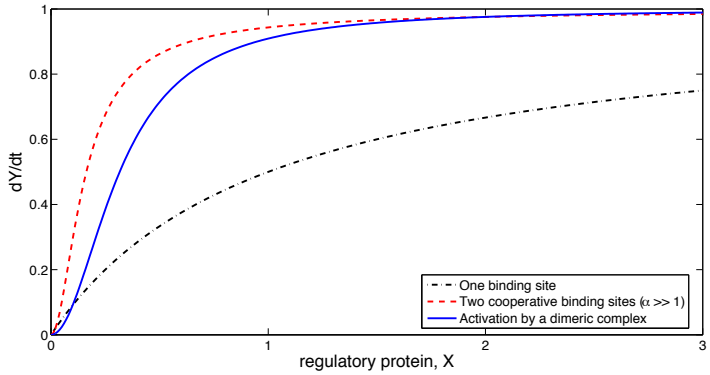


Figure 47: Comparison of cooperative binding sites (eq. 192, red curve) and activation by a homodimeric complex (eq. 202, blue curve).

Generalization

The generalization to the formation multimer (n-mer) yields to a Hill-like curve as in the case of cooperative binding sites (only the interpretation of the constant K is different):

$$\frac{dY}{dt} = v_s \frac{X^n}{K^n + X^n} \tag{203}$$

3.6 Transcriptional inhibition with an inducer

In the first case, we have seen that the rate of transcription in the case of an activation by an activator X can be expressed as:

$$v \sim \frac{X}{K_1 + X} \tag{204}$$

where $\frac{X}{K_1 + X}$ can be interpreted as the probability of the promoter to be active (i.e. bound to X).

We can derive the transcription rate in the case where X acts as a repressor in a similar way.

Assuming the following reaction scheme

$$\begin{gathered} \xrightarrow{k_s} \mathrm{X} \xrightarrow{k_d} \\ \mathrm{X} + \mathrm{D}_0 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{D}_1 \\ \mathrm{D}_0 + \mathrm{P} + \{y_i\} \xrightarrow{k_t} \mathrm{D}_0 + \mathrm{P} + \mathrm{Y} \end{gathered} \tag{205}$$

with the quasi-steady state assumption

$$\frac{dD_0}{dt} = 0 \tag{206}$$

we find

$$\frac{dY}{dt} = v_s \frac{K_1}{K_1 + X} \tag{207}$$

The term $\frac{K_1}{K_1 + X}$ is the probability that the promoter is active, i.e. not bound to the repressor X.

Now, let's consider that S can bind X to form a complex XS. S is an *inducer* since its binding to X prevents the latter to bind, and thereby to inhibit the promoter.

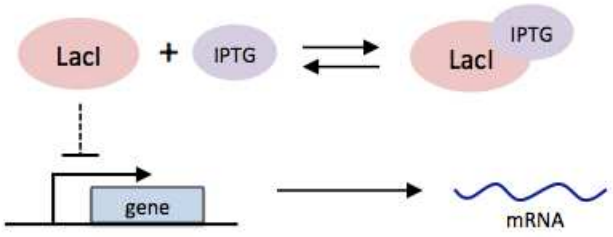


Figure 48: Inducer.

$$\mathrm{S} + \mathrm{X}_0 \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \mathrm{XS} \tag{208}$$

Assuming that the total concentration of X, X_T, is constant, the evolution of XS is described by

$$\frac{dXS}{dt} = k_a X.S - k_d XS \tag{209}$$

At steady state, $dXS/dt = 0$ and thus $K_S.XS = X.S$ or $XS = \frac{X_T S}{S + K_S}$ where $K_S = \frac{k_d}{k_a}$.

Thus, the level of effective inhibitor is

$$\begin{aligned} X^* &= X_T - \frac{X_T S}{S + K_S} \\ &= \frac{X_T K_S}{S + K_S} \end{aligned} \tag{210}$$

As expected the level of effective inhibitor X^* decreases when the level of the inducer S increases.

The transcription rate, in presence of an inhibitor and an inducer, then become:

$$\begin{aligned} \frac{dY}{dt} &= v_s \frac{K_1}{K_1 + X^*} \\ &= v_s \frac{K_1}{K_1 + \frac{X_T K_S}{S + K_S}} \end{aligned} \tag{211}$$

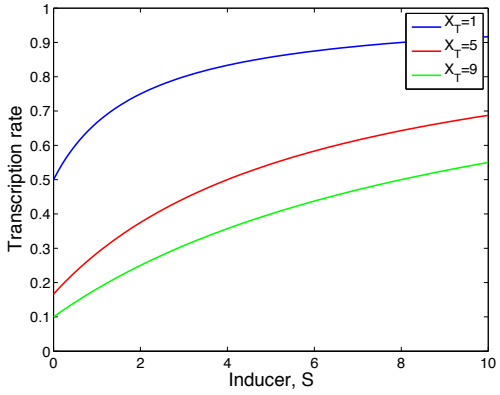


Figure 49: Transcription rate in presence of an inhibitor X_T and an inducer S (eq. 211). Note that when there is no inducer ($S = 0$), the transcription still takes place, but at a lower rate. Parameter values are: $v_s = 1$, $K_1 = 1$, $K_S = 1$.

3.7 Combining transcriptional activation and inhibition

Many genes are regulated by more than one transcription factor. The combined effects of these regulators can be described by a "multi-dimentional transcription function" (cf Alon's book). As an example let us examine a simple case in which a gene is regulated by a activator X and an repressor Y. How can these two regulators work together?

A common situation is that the activator and the repressor bind the promoter independently o two different sites (fig. XX). There are thus four binding states of promoter D: D, DX, DY, DXY, where DXY means that both X and Y are bound to the promoter. Transcription occurs mainly from the state DX in which the activator but not the repressor is bound. In the following we use the variables X and Y to denote the active forms of these regulator, i.e. X* and Y*.

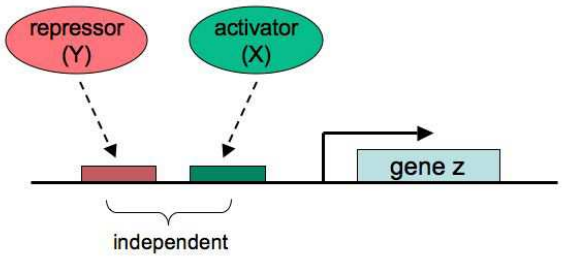


Figure 50: Gene expression can be controlled by several regulator.

The probability that X is bound is given by the Michaelis-Menten function (see above):

$$P(\text{X bound}) = \frac{X}{K_1 + X} = \frac{X/K_1}{1 + X/K_1} \tag{212}$$

The probability that Y is not bound is given by the Michaelis-Menten function (see above):

$$P(\text{Y not bound}) = 1 - \frac{Y}{K_2 + Y} = \frac{1}{1 + Y/K_2} \tag{213}$$

Since the two binding events are independent, the probability that the promoter D is bound to X and not to Y is given by the product of the two probabilities:

$$\begin{aligned} P(\text{X bound \& Y not bound}) &= P(\text{X bound}).P(\text{Y not bound}) \\ &= \frac{X/K_1}{1 + X/K_1} \frac{1}{1 + Y/K_2} \\ &= \frac{X/K_1}{1 + X/K_1 + Y/K_2 + XY/K_1K_2} \end{aligned} \tag{214}$$

and the output promoter activity is given by the production rate b times the probability:

$$v = b \frac{X/K_1}{1 + X/K_1 + Y/K_2 + XY/K_1K_2} \tag{215}$$

This results in an "X AND NOT Y" transcription function.

In many promoters , when the repressor binds, repression is only partial and there is basal transcription (leakage). In such case, the state in which both X and Y bind, DXY also contributes to the transcription rate, with $b' < b$, to the promoter activity:

$$v = \frac{bX/K_1 + b'XY/K_1K_2}{1 + X/K_1 + Y/K_2 + XY/K_1K_2} \tag{216}$$

This results in an input function with three plateau levels: zero when $X = 0$, b when X is high and Y low, and b' when both X and Y are high. This continuous input function can be approximated by a logic function:

$$v = \theta(X > K_1)(b(1 - \theta(Y > K_2)) + b'\theta(Y > K_2) \tag{217}$$

where θ is the step function, equal to 0 (if its argument is false) or 1 (if its argument id true).

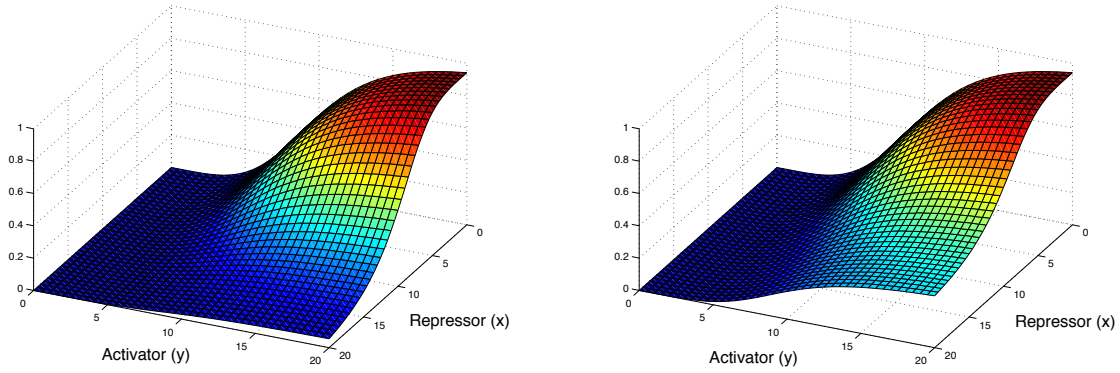


Figure 51: Transcription rate function in the presence of an activator and an inhibitor. Left: $b_1 = 1$, $b_2 = 0$, $b_2 = O$, $K_1 = 10$, $K_2 = 10$, and $n = 4$. Right: idem except $b_2 = 0.3$.

These results can be generalized. The transcription rate function can often be described by the ratio of polynomials of the active concentrations of the transcription factors X_i, with $i = 1, 2, ...n$. For example,

$$v = \frac{\sum_i b_i (X/K_i)^{n_i}}{1 + \sum_i b_i (X/K_i)^{m_i}} \tag{218}$$

The parameter K_i is the activation or repression coefficient for the transcription factor X_i,while b_i is its maximal contribution to expression, and the Hill coefficients are $n = m$ for activation and $n = 0, m > 0$ for repression. These types of functions have been found suitable to describe experimentally determined input function (Setty et al, 2003). More sophisticated epxression are also possible if the transcription factors interact with each other at the protein level (Buchler et al 2003).

4 Appendix

4.1 Reaction in series - derivation

We detail here how to solve the evolution equations for the reactions in series (see reactional scheme 29):

$$\frac{dA}{dt} = -k_1 A \tag{219}$$
$$\frac{dB}{dt} = k_1 A - k_2 B \tag{220}$$
$$\frac{dC}{dt} = k_2 B \tag{221}$$

Integrating the evolution equation for A is straightfoward. Assuming that initially $A(0) = A_0$, we find

$$A(t) = A_0 e^{-k_1 t} \tag{222}$$

The evolution equation for B then writes

$$\frac{dB}{dt} = k_1 A_0 e^{-k_1 t} - k_2 B \tag{223}$$

which can be rearranged as:

$$\frac{dB}{dt} + k_2 B = k_1 A_0 e^{-k_1 t} \tag{224}$$

This can be solved by multiplying both sides by the integrating factor $e^{k_2 t}$:

$$\frac{dB}{dt} e^{k_2 t} + k_2 B e^{k_2 t} = k_1 A_0 e^{-k_1 t} e^{k_2 t} \tag{225}$$

$$\frac{d}{dt}\left(B e^{k_2 t}\right) = k_1 A_0 e^{(k_2 - k_1)t} \tag{226}$$

Then, after integration, we find:

$$\begin{aligned} B e^{k_2 t} &= \int k_1 A_0 e^{(k_2 - k_1)t} dt \\ &= k_1 A_0 \frac{1}{k_2 - k_1} e^{(k_2 - k_1)t} + K \qquad (227) \\ &= \frac{k_1}{k_2 - k_1} A_0 e^{(k_2 - k_1)t} + K \qquad (228) \end{aligned}$$

Assuming that initially $B(0) = 0$, we find that the integration constant K is

$$K = -A_0 \frac{k_1}{k_2 - k_1} \tag{229}$$

Thus

$$B(t) = \frac{k_1}{k_2 - k_1} A_0 e^{(k_2 - k_1)t} e^{-k_2 t} - A_0 \frac{k_1}{k_2 - k_1} e^{-k_2 t} \tag{230}$$

$$B(t) = \frac{k_1 A_0}{k_2 - k_1} \left(e^{-k_1 t} - e^{-k_2 t} \right) \tag{231}$$

To solve the evolution equation for C, we then need to integrate

$$\frac{dC}{dt} = k_2 \frac{k_1 A_0}{k_2 - k_1} \left(e^{-k_1 t} - e^{-k_2 t} \right) \tag{232}$$

i.e.

$$\int dC = k_2 \int \frac{k_1 A_0}{k_2 - k_1} \left(e^{-k_1 t} - e^{-k_2 t} \right) dt \tag{233}$$

$$C = k_2 \frac{k_1 A_0}{k_2 - k_1} \left(-\frac{1}{k_1} e^{-k_1 t} + \frac{1}{k_2} e^{-k_2 t} \right) + K' \tag{234}$$

Assuming that initially $C(0) = 0$, we find that the integration constant K' is

$$K' = -A_0 \tag{235}$$

Thus

$$C(t) = A_0 \left(1 - \frac{1}{k_2 - k_1} \left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t} \right) \right) \tag{236}$$

4.2 Quasi-steady state approximation

In this appendix we demonstrate how the fact that the enzyme is much lower than the substrate leads to the approximation that the concentration of the complex C does not change significatively with time, i.e. that:

$$E << S \Rightarrow \frac{dC}{dt} \simeq 0 \tag{237}$$

First, we define dimensionless variable as follows:

$$s = \frac{S}{S_0},\ e = \frac{E}{E_T},\ \text{and}\ c = \frac{C}{E_T} \tag{238}$$

we also define a new time:

$$\tau = k_1 E_T t \tag{239}$$

Because the total concentration in enzyme is fixed ($E + C = E_T$), we have

$$e + c = 1 \tag{240}$$

We start by expressing dS/dt in terms of dimensionless variables. With the definitions (238),

$$\frac{dS}{dt} = -k_1 SE + k_{-1} C \tag{241}$$

becomes

$$\frac{ds}{d\tau} = -se + \alpha c = -s + c(s + \alpha) \tag{242}$$

where

$$\alpha = \frac{k_{-1}}{k_1 S_0}$$

Similarly, the evolution equation for C,

$$\frac{dC}{dt} = k_1 SE - (k_{-1} + k_2) C \tag{243}$$

becomes

$$\frac{E_T}{S_0}\left(\frac{dc}{d\tau}\right) = se - \frac{\beta}{k_1 S_0} c = s(1 - c) - \frac{\beta}{k_1 S_0} c \tag{244}$$

where

$$\beta = k_{-1} + k_2$$

The hypothesis that the enzyme is much lower than the substrate can be expressed as:

$$\epsilon = \frac{E_T}{S_0} << 1 \tag{245}$$

Expressing ϵ in eq. 244 we get

$$\epsilon \frac{dc}{d\tau} = s - c\left(s + \frac{\beta}{k_1 S_0}\right) \tag{246}$$

At the limit

$$\epsilon \to 0 \tag{247}$$

we have

$$\epsilon \frac{dc}{d\tau} = s - c\left(s + \frac{\beta}{k_1 S_0}\right) \simeq 0 \tag{248}$$

From eq. 248, this we deduce:

$$c = \frac{s}{\gamma + s} \tag{249}$$

where

$$\gamma = \frac{\beta}{k_1 S_0}$$

and, going back to the original variable, we find

$$C = \frac{E_T S}{K_m + S} \tag{250}$$

where

$$K_m = \frac{\beta}{k_1} = \frac{k_{-1} + k_2}{k_1}$$

which is the michaelian constant.

Restarting now from eq 241, where we replace c by its expression (eq. 249) we obtain

$$\frac{ds}{d\tau} = -s + \frac{s}{\gamma + s}(s + \alpha) = \frac{s(\alpha - \gamma)}{\gamma + s} \tag{251}$$

We see that:

$$\alpha - \gamma = -\frac{k_2}{k_1 S_0} \tag{252}$$

and therefore

$$\frac{ds}{d\tau} = -\frac{k_2}{k_1 S_0}\frac{S}{K_m + S} \tag{253}$$

$$\frac{dS}{d(k_1 E_T t)} = -\frac{k_2}{k_1}\frac{S}{K_m + S} \tag{254}$$

$$\frac{dS}{dt} = -k_2 \frac{ES}{K_m + S} = -V_{max}\frac{S}{K_m + S} \tag{255}$$

Hence, the rate at which the substrate descreases is equal to the rate at which the product appears

$$-\frac{dS}{dt} = \frac{dP}{dt} = v \tag{256}$$

and

$$v = V_{max}\frac{S}{K_m + S} \tag{257}$$

where

$$K_m = \frac{k_{-1} + k_2}{k_1} \text{ and } V_{max} = k_2 E$$

Note that

$$k_2 \to 0 \Rightarrow K_m \to K_S = \frac{k_{-1}}{k_1} \tag{258}$$

When the reaction is fast, K_M tends to the equilibrium constant of the first reaction.

If $S << K_m$, we observe a first order kinetics (linear relation between v and S):

$$v = kS \tag{259}$$

where

$$k = \frac{V_{max}}{K_m}$$

If $S >> K_m$, we observe a zero-order kinetics (constant rate v):

$$v = V_{max} \tag{260}$$

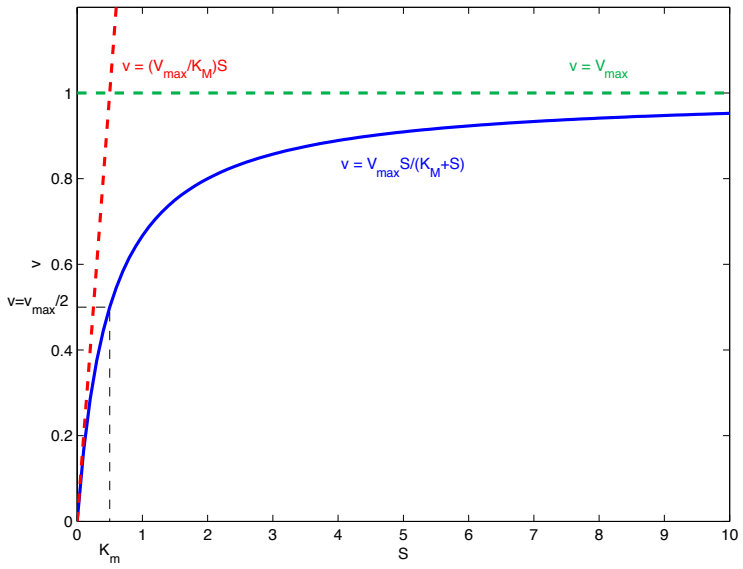


Figure 52: Michaelis-Menten kinetics

4.3 Validity of the quasi-steady state approximation

In deriving the Michaelis-Menten equation, it was assumed that a steady state would be reached in which $dC/dt = 0$. In fact Eq. for dC/dt in Eqs. (52) is readily integrable if S is treated as a constant, and it is instructive to derive a rate equation without making the steady state assumption, because this sheds ligt on the validity of the assumption (Cornish-Bowden, 1995, p. 29). Separating the two variables C and t, we have

$$\int \frac{dC}{k_1 E_T S - (k_1 S + k_{-1} + k_2)C} = \int dt \tag{261}$$

Integrating both sides, we find:

$$\frac{\ln[k_1 E_T S - (k_1 S + k_{-1} + k_2)C]}{-(k_1 S + k_{-1} + k_2)} = t + \alpha \tag{262}$$

At the instant when the reation starts, there has not been enough time to produce any ES complex, i.e. $C = 0$ when $t = 0$ and hence:

$$\alpha = \frac{\ln(k_1 E_T S)}{-(k_1 S + k_{-1} + k_2)} \tag{263}$$

Thus,

$$\ln\left[\frac{k_1 E_T S - (k_1 S + k_{-1} + k_2)C}{k_1 E_T S}\right] = -(k_1 S + k_{-1} + k_2)t \tag{264}$$

Taking exponentials of both sides, we have

$$1 - \frac{(k_1 S + k_{-1} + k_2)C}{k_1 E_T S} = e^{-(k_1 S + k_{-1} + k_2)t} \tag{265}$$

and solving for C we have

$$C = \frac{k_1 E_T S[1 - e^{-(k_1 S + k_{-1} + k_2)t}]}{k_1 S + k_{-1} + k_2} \tag{266}$$

The rate is given by $v = k_2 C$, and thus, substituting $v_m ax = k_2 E_T$ and $K_M = (k_{-1} + k_2)/k_1$, we have:

$$v = \frac{v_{max} S[1 - e^{-(k_1 S + k_{-1} + k_2)t}]}{k_M + S} \tag{267}$$

When t becomes very large the exponential term approaches 0 and Eqs. (267) becomes identical to the Michaelis-Menten Eq. (61). How large t must be for this to happen depends on the magnitude of $(k_1 S + k_{-1} + k_2$. If it is of the order of 1000 s^{-1} (a reasonable value in practice), then the exponential term is less than 0.01 for value greater that 5 ms. In other words Eq. (267) should become indistinguishable from the Michaelis-Menten equation after a few millisconds.

4.4 Comparison of developed vs compact Michaelis-Menten kinetics

Numerical simulation of the "developed" reactional scheme (Eqs. 52) and the Michaelis-Menten kinetics (eq. 61) shows a very good agreement (Fig. 53).

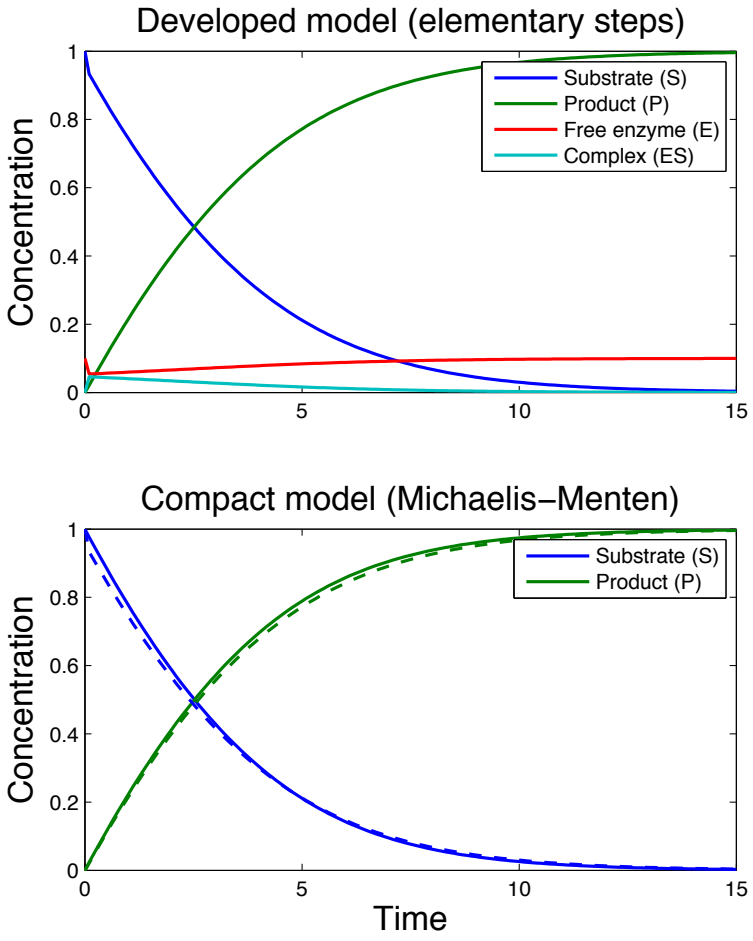


Figure 53: Comparison of developed vs compact Michaelis-Menten kinetics. Parameter values: $S_0 = 1$, $P_0 = 0$, $E_T = 0.1$, $k_1 = 50$, $k_{-1} = 50$, $k_2 = 5$, $K_M = (k_{-1} + k_2)/k_1$, $v_{max} = k_2 E_T$.

4.5 Examples of kinetic values

Enzyme reaction rates

Table 1 gives some examples of kinetics values for enzyme reactions.

Enzyme	Reaction	$K_M(M)$	k_2 (s^{-1})	k_2/K_M ($s^{-1}M^{-1}$)
Chymotrypsin	proteins degradation	$1.5\ 10^{-2}$	0.14	9.3
Pepsin	proteins degradation	$3\ 10^{-4}$	0.5	$1.7\ 10^{3}$
Ribonuclease	RNA degradation	$7.9\ 10^{-3}$	$7.9\ 10^{2}$	$1\ 10^{5}$
Fumarase	fumarate $\rightarrow$ malate	$5\ 10^{-6}$	$8\ 10^{2}$	$1.6\ 10^{8}$
β-galactosidase	lactose hydrolysis	$4\ 10^{-3}$	$4.8\ 10^{2}$	$1.2\ 10^{5}$
Acetylcholinesterase	acetylcholine hydrolysis	$9\ 10^{-5}$	$1.4\ 10^{4}$	$1.6\ 10^{8}$

Table 1: Examples of kinetic values. The ratio k_2/K_M is a measure of how efficiently an enzyme converts a substrate into a product. Source: wikipedia.

Transcription / Translation rates

The rate of transcription in mammalian cells is of the order of 1000 nucleotides/minute (Hargrove et al. 1991). Therefore, a mRNA of 1 kb is produced every minute. If we consider a cellular volume of $10\ 10^{-12}$ L and a concentration of 10nM, this corresponds to a transcription rate of about 0.1 nM/h.

The rate of translation (protein synthesis) is of the order of 140 amino acids/minute (Lewin, 1997). Therefore, a protein of 1000 aa is produced every 10 minutes. If we consider a cellular volume of $10\ 10^{-12}$ L and a concentration of 10nM, this corresponds to a translation rate of about 0.01 nM/h.

4.6 Competitive inhibition

We show here how to derive the enzyme kinetics equation in presence of competitive inhibition. The cases of other types of inhibition can be treated in a similar way.

The reaction scheme is:

$$\begin{array}{l} \mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_2}{\rightarrow} \mathrm{E} + \mathrm{P} \\ \mathrm{E} + \mathrm{I} \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} \mathrm{EI} \end{array} \tag{268}$$

First, let's define the equilibrium constant

$$K_I = \frac{k_{-i}}{k_i} \tag{269}$$

Thus, assuming that the binding of the inhibitor is at equilibrium:

$$k_i E * I = k_{-i} EI \tag{270}$$

$$EI = \frac{E * I}{K_I} \tag{271}$$

Using the quasi-steady state assumption

$$\frac{dES}{dt} = k_1 E * S - k_{-1} ES - k_2 E * S \underbrace{-k_i E * I + k_{-i} EI}_{=0} \tag{272}$$

$$= k_1 E * S - k_{-1} ES - k_2 E * S = 0 \tag{273}$$

we find

$$k_1 E * S = (k_{-1} + k_2) ES$$

$$E = \frac{(k_{-1} + k_2) ES}{k_1 S} \tag{274}$$

Then, combining eqs. (271) and (274), we get

$$\begin{aligned} E_{tot} &= ES + E + EI \\ &= ES + E + \frac{E * I}{K_I} \\ &= ES + E(1 + \frac{I}{K_I}) \\ &= ES + \frac{(k_{-1} + k_2) ES}{k_1 S}(1 + \frac{I}{K_I}) \\ &= ES \left(1 + \frac{(k_{-1} + k_2)}{k_1 S}(1 + \frac{I}{K_I})\right) \end{aligned} \tag{275}$$

Finally the rate of production of the product P is

$$\begin{aligned}\frac{dP}{dt} &= k_2 * ES \\ &= k_2 \frac{E_{tot}}{1 + \frac{(k_{-1}+k_2)}{k_1 S}(1 + \frac{I}{K_I})} \\ &= \frac{k_2 E_{tot} S}{S + \frac{(k_{-1}+k_2)}{k_1}(1 + \frac{I}{K_I})}\end{aligned} \tag{276}$$

Defining:

$$v_{max} = k_2 E_{tot} \tag{277}$$

$$K_M = \frac{(k_{-1}+k_2)}{k_1} \tag{278}$$

Eq. (276) can be rewritten:

$$v = v_{max} \frac{S}{K_M \left(1 + \frac{I}{K_I}\right) + S} \tag{279}$$

4.7 A rapid (but approximative) derivation of the Hill function

A less rigorous way to derive the Hill a function is to consider that n substrate molecules binds together to form a n-mer complex, which, in turn, binds the enzyme E:

$$\begin{aligned} \mathrm{n\ S} &\overset{k_a}{\underset{k_d}{\rightleftharpoons}} \mathrm{S}_n \\ \mathrm{S}_n + \mathrm{E} &\overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} \mathrm{ES}_n \overset{k_2}{\rightarrow} \mathrm{E + n\ P} \end{aligned} \tag{280}$$

At equilibrium, $\dfrac{S^n}{S_n} = \dfrac{k_d}{k_a} = K_d$.

The second set of reactions follows a Michaelis-Menten kinetics:

$$\begin{aligned} v = \frac{dP}{dt} &= v_{max} \frac{S_n}{K_s + S_n} \\ &= v_{max} \frac{S^n}{K^n + S^n} \end{aligned} \tag{281}$$

with $v_{max} = k_2 E_{tot}$, $E_{tot} = E + ES_n$, $K_s = \dfrac{k_1}{k_{-1} + k_2}$, and $K = \sqrt[n]{K_d K_s}$.

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