Mathematical Modelling for Systems Biology

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Contents

Chapter 1

Biochemical Reaction Modelling

This chapter provides an introduction to the formulation and analysis of differential-equation-based models for biological regulatory networks. We will discuss basic reaction types and the use of mass action kinetics and of simplifying approximations in the development of models for biological signaling.

1.1 An Introduction to Modelling

The cell is a large dynamic system with thousands of interacting components. To predict how a dynamical system evolves over time and what equilibrium it assumes we can formulate differential equations that describe the state of the system. Each system component is represented by a state variable, x. Typically x is a concentration, but x could also represent a density or the number of molecules. The change of x, Δx, per time interval Δt depends on the rate v_+ at which x is generated, the "gain" rate, and on the loss rate v_- at which x is removed. Here the rate of generation reflects all processes that lead to an increase in x, i.e. synthesis, change of chemical modification and many more while the loss rate includes all processes that lead to a decrease in the value of x. More formally we can write

$$\frac{\Delta x}{\Delta t} = \text{gain rate - loss rate} = v_+ - v_-. \tag{1.1}$$

Instead of considering finite time intervals Δt we will consider the change in an infisitimal small time interval dt, such that

$$\frac{dx}{dt} = \text{gain rate - loss rate} = v_+ - v_-. \tag{1.2}$$

In many cases there are multiple components and multiple compartments (i.e. cytoplasm and nucleus) with distinct pools of x so that instead of a single x we have many state variables $x_i \in \{x_1(t), x_2(t), \ldots\}$. The value of each x_i then also changes due to the formation of complexes and due to shuttling between compartments. We then write for

a change of each state variable $x_i(t)$ in this time interval dt

$$\frac{dx_i}{dt} = \text{synthesis - degradation} \pm \text{shuttling} \pm \text{complex formation} \pm \text{chemical modification} \pm \cdots \tag{1.3}$$

The values of all state variables at a given time point t constitute the state of the system at time t. When gain and loss rates balance the variable no longer changes with time, i.e. $\frac{dx_i}{dt} = 0$. When the gain and loss rates of all variables balance then the system reaches an equilibrium point, also referred to as steady state or fixed point. In general, the gain and loss rates change as the values of the state variables change. This is the basis of all feedback regulation. Mathematically, we say that the system of ODEs is coupled, i.e. the differential equations for the different variables depend on each other

$$\frac{dx_i}{dt} = f(x_1, x_2, ..., x_n). \tag{1.4}$$

This means that we need to consider the entire set of equations simultaneously and cannot solve the different equations separately. Software packages (such as Matlab or Mathematica) are available that provide algorithms to solve these sets of equations numerically. In the following we will discuss how the rates of some typical biological reaction types are affected by changes in the values (i.e. concentrations) of state variables.

1.2 Basic Reaction Types

The most accurate model can be obtained when the law of mass action is used to formulate kinetic laws for all elementary reactions in Eq. 1.3.

> **Mass Action Kinetics** According to the law of mass action, the rate of a reaction is proportional to the probability of a collision of reactants. This probability, in turn, is proportional to the concentrations of the participating molecules to the power of the molecularity, i.e. the number in which they enter the specific reaction.

Thus, if the molecules participating in the gain reaction have concentrations c_i and molecularites m_i, then the gain rate according to the general mass action rate law is given by

$$v_+ = k_+ \prod_i c_i^{m_i}. \tag{1.5}$$

Similarly, if the molecules participating in the loss reaction have concentrations c_j and molecularites m_j, then the loss rate according to the general mass action rate law is given by

$$v_- = k_- \prod_j c_j^{m_j}. \tag{1.6}$$

The equilibrium constant K_{eq} is then given by

$$K_{eq} = \frac{k_+}{k_-} = \frac{\prod_j c_j^{m_j}}{\prod_i c_i^{m_i}} \tag{1.7}$$

where the concentrations are those in equilibrium. Here, it should be noted that this only holds as long as there are no parallel inputs to the gain or loss rates. Parallel inputs would have to be included as sums, i.e.

$$v_+ = \sum_j v_{+j} = \sum_j k_{+j} \prod_i c_i^{m_{ji}}. \tag{1.8}$$

We will continue with the simple version. Here, we can distinguish between different order reactions and relate these to the basic reaction types that are frequently found in biological settings. Thus, for

$$\frac{dc_i}{dt} = kc_i^{m_i} \tag{1.9}$$

the order of the reaction depends on m_i. For $\sum_i m_i = 0$, we speak of a zero-order reaction, for $\sum_i m_i = 1$ of a first-order reaction, and for $\sum_i m_i = 2$ of a second order reaction.

1.2.1 0th Order Reactions - Constant Reaction Rates

0th order reactions are the simplest of all reactions because the rate of the reaction does not depend on the state variables. This kinetic law is used frequently to describe the synthesis of a molecular component.

Constant Synthesis Assuming that the species X is produced at a constant rate k_{prod} we write for the concentration of X, $[X]$,

$$\frac{d[X]}{dt} = k_{prod}. \tag{1.10}$$

This equation can be solved as $[X(t)] = [X(t_0)] + k_{prod}(t - t_0)$ and we note that the concentration of X at time t depends only on the initial value of X at time t_0 and on the time interval $t - t_0$ that has passed. Accordingly, the rate at which X is produced does not change when the concentration of X is changed (Fig. 1.1A, a).

MATLAB Exercise

Simulate equation 1.10 with $k_{prod} = 1$ and $[X(t_0)]$=0 for $t \in [0, 10]$ and compare the solution to the analytical solution:

```
function ODE_model1_prod()
x0 = 0; % initial concentration
```

```
k_prod = 1;
tspan = [0, 10];

[t,x] = ode15s(@rhs_prod, tspan, x0, [], k_prod);
plot(t, x, 'r.', t, x0+k_prod*t, 'k-')
xlabel('Time t')
ylabel('Concentration x')
legend('numerical solution', 'analytical solution')
end


function dxdt = rhs_prod(t,x,p0)
    dxdt = [p0];
end
```

1.2.2 1st Order Reactions - Monomolecular Reactions

Most biological reactions are catalyzed or affected by components whose concentrations vary with time. Reactions that only depend on one such component are referred to as monomolecular reactions. Important examples include the decay of a molecular species or its transport between compartments, i.e. cytoplasm and nucleus. Mathematically the dynamics of the state variable linearly depends on the state variable in 1st order reactions.

Linear Degradation The rate at which a protein, mRNA or similar is removed or inactivated is often proportional to its own abundance, i.e. it changes linearly with its own concentration (Fig. 1.1A, b). We write for the concentration of such a component X

$$\frac{d[X]}{dt} = -k_{deg}[X]. \tag{1.11}$$

This equation can be solved as $[X(t)] = [X(t_0)]\exp(-k_{deg}(t-t_0))$ and we note that the concentration of X decays exponentially over time. An important measure is the characteristic time $t_{1/2} = \frac{\ln(2)}{k_{deg}}$ by which the initial concentration $[X(t_0)]$ has decreased by half.

MATLAB Exercise

Simulate equation 1.11 with $k_{deg} = 1$ and $[X(t_0)]$=1 for $t \in [0,10]$ and compare the solution to the analytical solution:

```
function ODE_model2_degradation()
x0 = 1; % initial concentration
k_deg = 1;
tspan = [0, 10];

[t,x] = ode15s(@rhs_prod, tspan, x0, [], k_prod);
plot(t, x, 'r.', t, x0 * exp(-k_deg*t), 'k-')
xlabel('Time t')
ylabel('Concentration x')
legend('numerical solution', 'analytical solution')
end


function dxdt = rhs_prod(t,x,p0)
    dxdt = [-p0*x];
end
```

Shuttling between Compartments Similarly the shuttling between two compartments (i.e. nucleus and cytoplasm) can be described by two coupled differential equations for the concentrations of X in the nucleus, $[X_n]$, and in the cytoplasm $[X_c]$. Importantly, we need to take the volume difference between the two compartments into account. Let us denote the different volumes of cytoplasm and nucleus as V_c and V_n. If a concentration $[X_c]$ shuttles from the cytoplasm to the nucleus then $V_c[X_c]$ molecules of X_c leave the cytoplasm and enter the nucleus. In the nucleus, these $V_c[X_c]$ molecules correspond to a concentration $V_c/V_n \cdot [X_c]$, where V_n is the volume of the nucleus. Accordingly, if X is exported from the nucleus at rate k_{out} and is imported from the cytoplasm at rate k_{in} then we have

$$\begin{aligned} \frac{d[X_n]}{dt} &= k_{in}\frac{V_c}{V_n}[X_c] - k_{out}[X_n] \\ \frac{d[X_c]}{dt} = -\frac{V_n}{V_c}\frac{d[X_n]}{dt} &= -k_{in}[X_c] + k_{out}[X_n]\frac{V_n}{V_c}. \end{aligned} \tag{1.12}$$

Since the total amount (NOT concentration) of X is conserved inside the cell, i.e. $[X_c]V_c + [X_n]V_n = T = const$, we can decouple these two ODEs. We can then write $[X_c] = \frac{T-[X_n]V_n}{V_c}$ and obtain a differential equation that is similar to Eq. 1.11 except for an additional constant term, $k_{in}\frac{T}{V_n}$,

$$\frac{d[X_n]}{dt} = k_{in}\frac{V_c}{V_n}\frac{T-[X_n]V_n}{V_c} - k_{out}[X_n] = k_{in}\frac{T}{V_n} - (k_{in}+k_{out})[X_n]. \tag{1.13}$$

MATLAB Exercise

Combine the previous MATLAB examples (production and liner decay) to simulate equation 1.13.

1.2.3 2nd Order Reactions - Bimolecular Reactions

Most reactions in biology involve some form of complex formation and therefore depend on the interaction of more than one time-varying component. Here it is important to distinguish between homo- and heterodimerization.

Complex formation - heterodimers The formation of heterodimers, XY, is the result of the interaction of two components X and Y (Fig. 1.1A, c). The reaction rate depends linearly on both the concentrations of X and of Y. Assuming that the reaction proceeds at rate k_{on} and that the total concentrations of both components is constant we have

$$\frac{d[XY]}{dt} = k_{on}[X][Y] = k_{on}(X_T - [XY])(Y_T - [XY]) \tag{1.14}$$

where $X_T = [X] + [XY]$ and $Y_T = [Y] + [XY]$ are the total concentrations of X and Y respectively. This equation can be solved to give $[X](t) = X_T - [XY(t)] = \frac{Y_T - X_T}{\frac{Y_T}{X_T}\exp((Y_T - X_T)kt) - 1}$.

MATLAB Exercise

Simulate equation 1.14 with $k_{on} = 1$, $[X_T] = [Y_T]$=1 and $[XY(0)]$=0 for $t \in [0, 10]$ and compare the solution to the analytical solution.

Complex formation - homodimers Similarly, the kinetics of homodimer formation between two X components can be described by the following quadratic rate law

$$\frac{d[X_2]}{dt} = k_{on}[X]^2 = k_{on}(X_T - 2[X_2])^2 \tag{1.15}$$

where X_T is the total amount of X which we again assume to be constant. Here the rate of homodimer X_2 formation depends non-linearly on the concentration of the monomers X (Fig. 1.1A, d). The dynamics of X can be described by

$$\frac{d[X]}{dt} = -2\frac{d[X_2]}{dt} = -2k_{on}[X]^2 \tag{1.16}$$

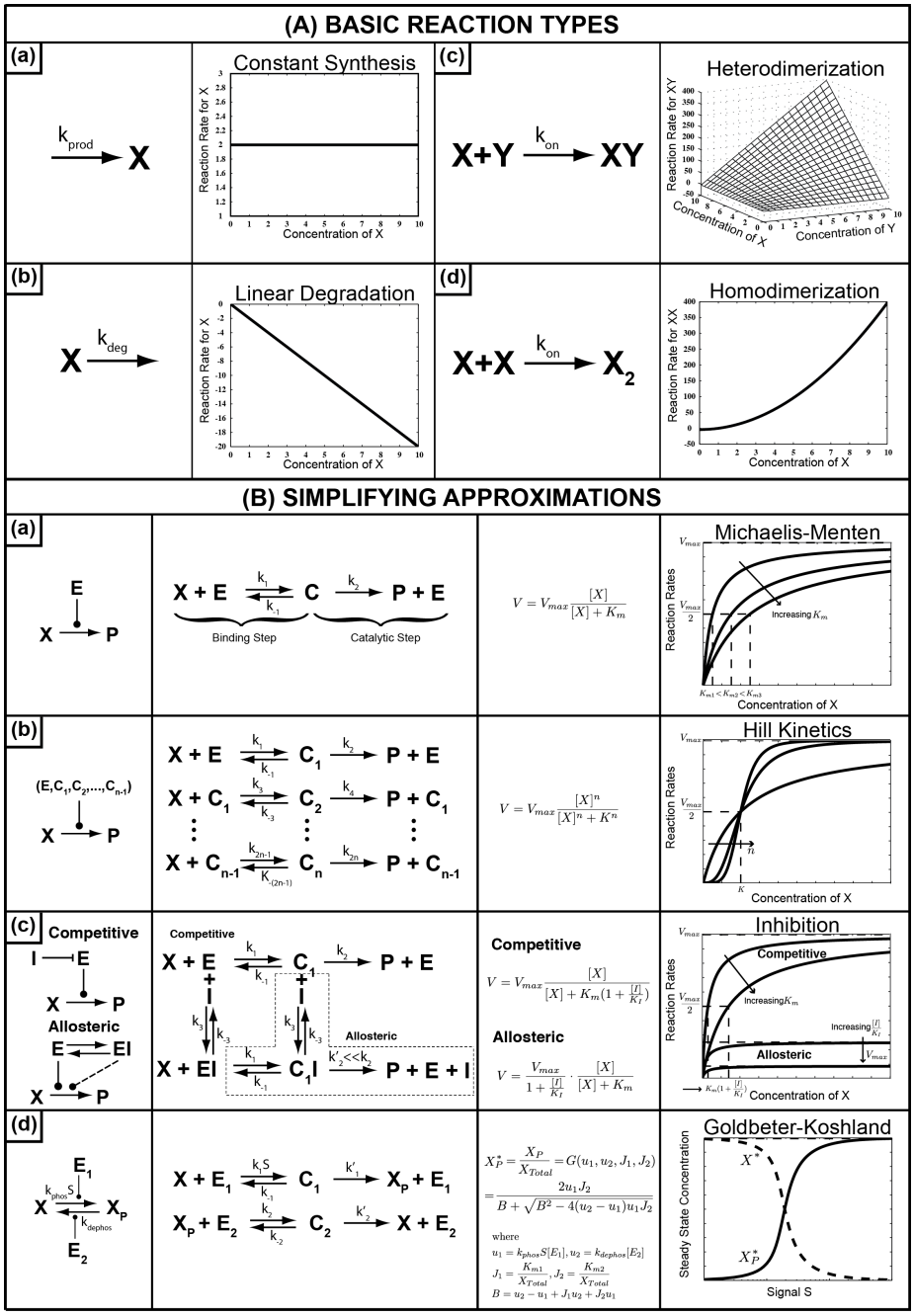

Figure 1.1: **Basic Reaction Types (A): (a)** Constant Synthesis. **(b)** Monomolecular Reactions: Linear Degradation. **(c)** Bimolecular Reactions: Heterodimer Formation. **(d)** Bimolecular Reactions: Homodimer Formation. **Simplifying approximations (B): (a)** Michaelis-Menten Kinetics. **(b)** Hill Kinetics. **(c)** Hill Kinetics with allosteric or competitive inhibition. **(d)** Goldbeter-Koshland Kinetics. The reaction scheme and a plot of the representative reaction rate versus the concentration of the reactant X (A) or the enzyme E (B) are depicted. In (B, d) the steady state concentrations of X_p and X are plotted versus the signal strength S.

The equation can be solved to give $[X](t) = \frac{X_T}{2X_T kt+1}$.

We note that there are many cases in which the total concentrations are not constant. The above simplification would then not apply and a set of coupled ODEs for the monomers and the dimers would then need to be solved. In case of higher order complexes the formation can, in general, be modeled as a sequential step of bimolecular reactions.

1.3 Rule-based modeling

Based on mass action, accurate models can be formulated even for large networks, as long as both the components (i.e. the proteins, compartments, complexes etc) and the rules and kinetics of their interactions are known. Typically such models are based on network cartoons of the form shown in Fig 1.2A. To translate such cartoons into mathematical models we assign a single state variable $x_i(t)$ to each icon. One state variable would be the unbound ligand $x_1(t)$. Another state variable would be the ligand-receptor complex, $x_2(t)$, and so on. The set of values of all state variables $\{x_1(t), x_2(t), ...\}$ at a given time point t constitutes the state of the system at time t.

In most biological networks, models based on mass action will lead to huge dynamic systems that are based on hundreds of ODEs to describe the interactions between less than 10 components, a problem referred to as "combinatorical complexity". The problem arises because proteins typically have multiple binding sites such that even a simple network as shown in Figure 1.2A translates into a much larger network as shown in Figure 1.2B when these are taken into account. The technical problem of generating such large system of ODEs can be overcome by rule-based modelling. The algorithm generates the system of ODEs from the formulated rules of interactions. Using only the set of sensible biochemical rules for what is known about our system it is easy to generate a comprehensive system and avoid making any errors due to missing interactions and/or unjustified assumptions. There are different software that have been designed to enable rule-based modeling. Among other softwares, most popular are `BioNetGen` (book find citation) and `Kappa` (Vincent Danos, missing citation...). The syntax and example below are based on the BioNetGen language (BNGL).

1.3.1 Rule-based modeling concepts

We can summarize the possible rules in rule-based modeling in the following five basic transformations. (1) Complex formation: a bond can be formed to link two molecules through their available binding sites. (2) Complex dissociation: an existing bond between two molecules can be removed (3) Change the state-label of a component: a molecule undergoes a certain post-translational state modification (e.g. become phosphorylated), or alters the state-label of its functional shape or conformation (e.g. open/closed conformation of integrins). (4) Add a molecule: production of a species. (5) Delete a molecule: degradation of a species. The above stated rules can be expressed as unidirectional transformations, but some of them can be also bidirectional (e.g. binding/unbinding, phosphorylation/de-phosphorylation, opening/closing).

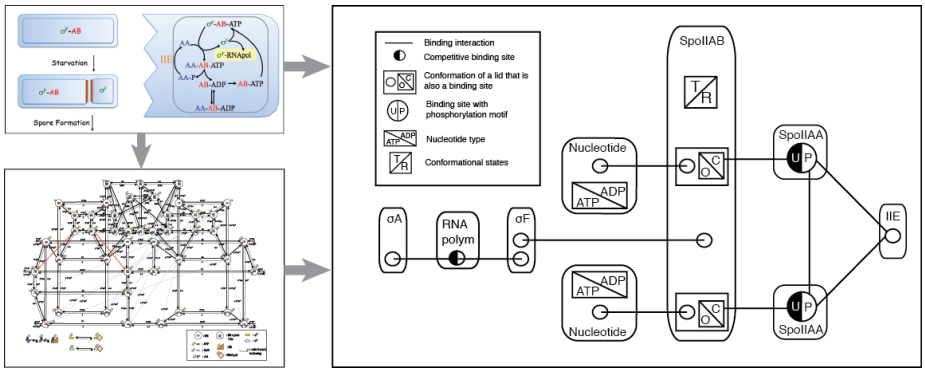

Figure 1.2: Examples of network representation. **(Up-left)** Cartoon of the regulatory interactions in the network that controls σ^F during sporulation in *Bacillus subtilis*. The image was reproduced from Figure 1 in [**?**]. **(Down-left)** Manually created network of interactions **(Right)** Contact map

Based on these concepts, already with a very small set of rules and molecule types we can generate enormous systems.

1.3.2 Contact maps

Large regulatory networks are difficult to visualise. On the one hand, the conceptual cartoons that are usually found in text books are too abstract to reflect the full system that needs to be modeled mathematically. On the other hand, detailed network maps are typically to dense to be readable. In rule-based modeling, contact maps are used. Contact maps depict the set of all possible interactions among the basic elements of the system; the actual transitions from reactants to products are not given explicitly. In Figure 1.2, an example of a signaling pathway is illustrated both in all three ways.

1.3.3 Simple example

The following figure illustrates some basic elements with a small example. Assume there are two interacting molecules, a ligand L and a receptor R. Let the ligand have one binding site for the receptor, and the receptor to have two binding sites, one for ligand-binding and one that enables homodimerization with another receptor. Also assume that the receptor has a tyrosine site Y with two state-labels, phosphorylated P and unphosphorylated U (Figure 1.3(a)). Having defined the molecules and their properties we can start defining patterns, e.g. free receptors irrespective of their state-label (Figure 1.3(b)). Now we can write down a set of simple rules that will summarize our set of interactions: the ligand and the receptor can form a heterodimer complex, this complex can further homodimerize, and this allows the receptors to further trans-phosphorylate each other (Figure 1.3(c)). This scenario of interactions could be further continued by just binding of adapter molecules on the phosphorylated site, or by enabling the modulation of other interactions upon phosphorylation.

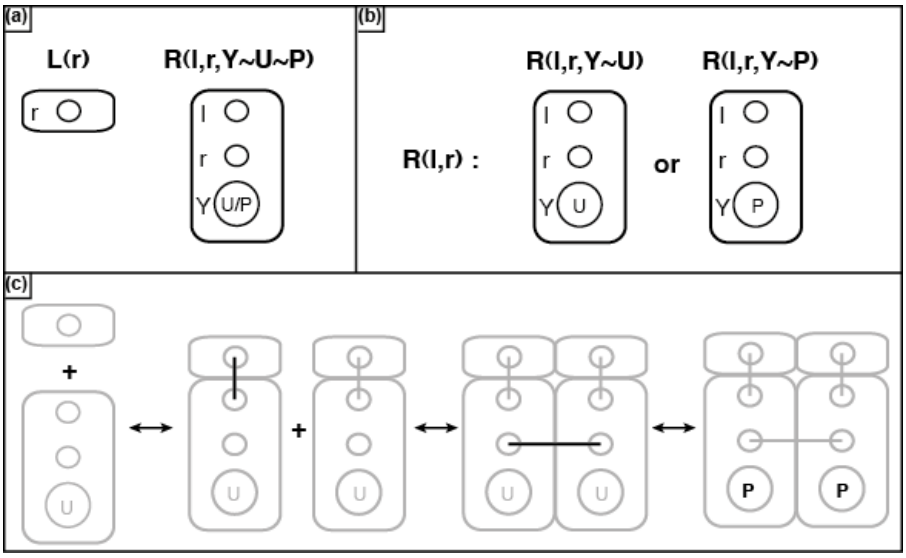

Figure 1.3: Rule-based modeling concepts using BioNetGen language (BNGL). **(a)** Molecules **(b)** Patterns **(c)** Simple example of interactions

1.4 Simplifying Approximations

If we formulate the kinetics of large networks based on first principles then the description becomes very complex and will be accurate only if we are able to determine a large number of parameters with high accuracy. In particular, in case of cooperative enzymes it can be very difficult to obtain accurate data on the reaction rates of all intermediate complexes. Most of the times we do not know all elementary/molecular interactions that regulate a particular reaction. Therefore there are many situations in which simplifications are sufficient and in fact preferable. Even from a computational point of view, it can make calculations more efficient.

1.4.1 Michaelis-Menten Kinetics

One frequently used approximation is quasi-stationarity of a reaction. Here the different time scales are exploited on which reactions proceed. If some reactions proceed much faster than others then certain concentrations are constant early on while other concentrations barely change at a later time. This is used in the derivation of Michaelis-Menten kinetics for the enzymatic turn-over of a substrate (Fig. 1.1B (a)). In a basic enzymatic reaction a substrate X binds to an enzyme E to form a substrate-enzyme complex C. Complex formation is a reversible reaction while the formation of the product P is irreversible,

$$X + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} E + P. \tag{1.17}$$

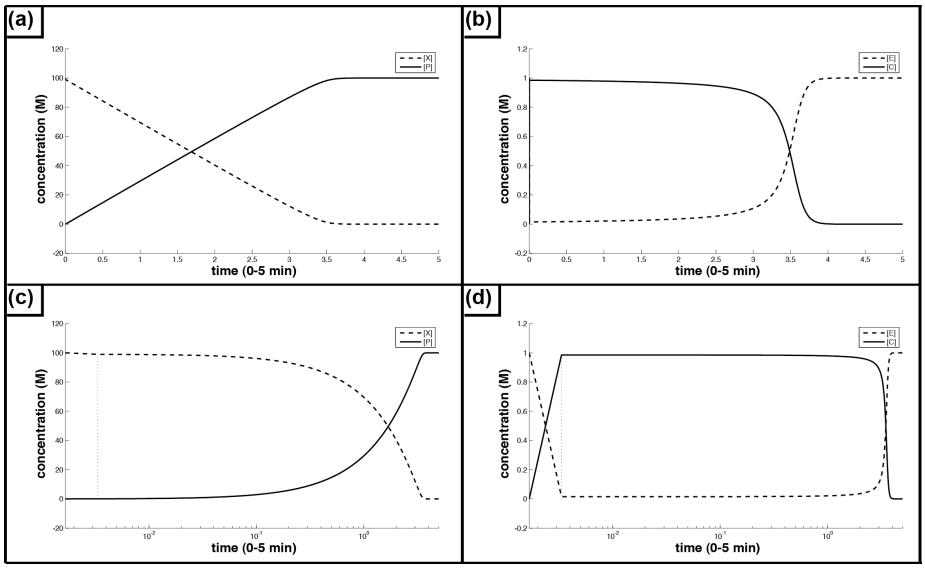

Figure 1.4: **The Kinetics of the Michaelis Menten Reaction (a, c)**The kinetics of substrate X and product P on linear and log scale. **(b,d)** The kinetics of enzyme E and substrate-enzyme complex C on linear and log scale.

The elementary reaction rates for the enzymatic turn-over of a substrate are:

$$\frac{d[X]}{dt} = k_{-1}[C] - k_1[X][E] \tag{1.18}$$

$$\frac{d[E]}{dt} = (k_{-1} + k_2)[C] - k_1[E][X] \tag{1.19}$$

$$\frac{d[C]}{dt} = -(k_{-1} + k_2)[C] + k_1[E][X] \tag{1.20}$$

$$\frac{d[P]}{dt} = k_2[C] \tag{1.21}$$

with initial conditions $[X(0)] = X_T$, $[E(0)] = E_T$, $[C(0)] = [P(0)] = 0$. We notice that $\frac{d[E]}{dt} + \frac{d[C]}{dt} = 0$, and thus $[E] + [C] = E_T$, i.e. the total amount of enzyme is conserved (Fig. 1.4B,D). Moreover, the differential equation for the product P is uncoupled from the other differential equations since P does not impact on X, C, or E. We can therefore reduce the set of 4 differential equations to a set of 2 coupled differential equations:

$$\frac{d[X]}{dt} = k_{-1}[C] - k_1[X](E_T - [C]) \tag{1.22}$$

$$\frac{d[C]}{dt} = -(k_{-1} + k_2)[C] + k_1(E_T - [C])[X] \tag{1.23}$$

Non-dimensionalization To simplify all subsequent analyses, we first non-dimensionalize the equations. As such, each variable and each parameter needs to be transformed into a dimensionless counterpart. There is no general rule as to how to non-dimensionalize. However, there are some guidelines: (1) If there is a maximal value that a variable can attain then it is sensible to normalize the variable with respect to this maximal value. We therefore write $s = \frac{[X]}{X_T}$, $c = \frac{[C]}{E_T}$. (2) Parameters should be grouped so as to reduce the total number of parameters. We write $\tau = k_1 E_T t$, $\kappa_1 = \frac{k_{-1}+k_2}{k_1 X_T}$, and $\kappa_2 = \frac{k_{-1}}{k_1 X_T}$. (3) If possible, very small and very large parameters should be generated so as to enable the use of perturbation methods. Here we exploit that the substrate concentration, X_T, is much larger than the total enzyme concentration, $[E_T]$ and thus $\varepsilon = \frac{E_T}{X_T} \ll 1$. We then obtain

$$\begin{aligned} \frac{ds}{d\tau} &= -s + c(s+\kappa_2) \\ \varepsilon \frac{dc}{d\tau} &= s - c(s+\kappa_1) \end{aligned} \tag{1.24}$$

with initial conditions $s(0) = 1$ and $c(0) = 0$.

Quasi-steady-state approximation A quasi-steady-state approximation can be used when processes occur on very different timescales such that within a certain time interval a variable barely changes in value. In case of the Michaelis-Menten model $\varepsilon \frac{dc}{d\tau} \approx 0$ once $\frac{dc}{d\tau} \leq O(1)$. In that case $\varepsilon \frac{dc}{d\tau} = s - c(s+\kappa_1) \approx 0$ and the quasi-state approximation thus yields as quasi-steady state $c = \frac{s}{s+\kappa_1}$. In dimensional form we then have $[C] = E_T \frac{k_1[X]}{k_1[X]+k_{-1}+k_2}$, and thus for the rate at which the product (P) is formed the well-known Michaelis-Menten kinetics

$$\frac{d[P]}{dt} = k_2[C] = k_2 E_T \frac{k_1[X]}{k_1[X]+k_{-1}+k_2} = v_{max} \frac{[X]}{[X]+K_m}. \tag{1.25}$$

$k_2 E_T$ is the maximal rate, v_{max}, at which this reaction can proceed when the substrate concentration is large ($[X] \gg K_m$). $K_m = \frac{k_{-1}+k_2}{k_1}$ is the Michaelis-Menten constant and specifies the substrate concentration at which the reaction proceeds at half-maximal rate. Importantly, the rate at which product is formed versus the substrate concentration yields a hyperbolic graph (Fig. 1.1B (a) RHS). While the conditions for Michaelis-Menten kinetics do not always strictly apply, such dependency of the reaction rate on the substrate concentration is observed more generally. In such cases the reaction rate v can be approximated by $v = v_{max} \frac{[X]}{[X]+K_m}$.

Note that this approximation is only valid at sufficiently long times. If we check the initial conditions we realize that we obtain a contradiction, i.e.

$$[C(t)] = [E_T] \frac{[X(t)]}{[X(t)]+K_m} \qquad \Rightarrow \qquad C(0) = 0 \neq E_T \frac{X_T}{X_T + K_m}. \tag{1.26}$$

There is thus an initial time interval in which C changes rapidly before assuming a relatively stable quasi-steady state value (Fig. 1.4D). We can estimate the length of

the relevant time scales. The first time scale T_c on which C changes rapidly while X remains about constant(Fig. 1.4D) can be estimated by setting $[X] = X_T$ in Eq. 1.23, i.e.

$$\begin{aligned} \frac{d[C]}{dt} &= -(k_{-1}+k_2)[C] + k_1(E_T - [C])X_T \\ \Leftrightarrow \frac{d[C]}{k_1(X_T+K_m)dt} &= -[C] + E_T\frac{X_T}{X_T+K_m}. \end{aligned} \tag{1.27}$$

With $[C](0) = 0$ this equation can be solved as

$$\Leftrightarrow [C](t) = E_T\frac{X_T}{X_T+K_m}\left(1-\exp\left(-k_1(X_T+K_m)t\right)\right). \tag{1.28}$$

The quasi-steady-state concentration of C, $[C]_{qstst} = E_T\frac{[X]}{[X]+K_m}$, is thus reached exponentially fast and

$$T_c = \frac{1}{k_1(X_T+K_m)} \tag{1.29}$$

represents the time within which the concentration of C reached its quasi-steady state value up to $1-\exp(-1) \sim 63\%$. This characteristic time can thus be used as the first time scale T_c over which $[C]$ is changing rapidly. The subsequent timescale on which X changes significantly can be estimated as

$$T_s = \frac{X_T}{|\frac{dX}{dt}|_{max}} \sim \frac{X_T+K_m}{k_2E_T}. \tag{1.30}$$

Here we used $d[X]/dt = -d[P]/dt = -k_2E_T\frac{[X]}{[X]+K_m}$ which applies once $[C]$ has reaches a quasi-steady state; the reaction speed is maximal initially while $[X] \sim X_T$.

While the quasi-steady state approximation yields the very useful Michaelis-Menten equation we may still want a full solution. It is not possible to get analytically a closed form solution for Eq. 1.24, but singular perturbation methods can be used to obtain approximate solutions.

Quasi-stationarity requires

1. a separation of the timescales on which C and X change rapidly, i.e. $T_c \ll T_s$ and thus
$$\frac{k_2 E_T}{k_1 (X_T + K_m)^2} \ll 1 \tag{1.31}$$
2. that the substrate concentration is almost constant in the time interval T_c, i.e. $\left|\frac{\Delta[X]}{X_T}\right| \ll 1$ with $|\Delta[X]| \sim k_1 E_T X_T T_c$ since in this time interval $[X]$ is changing mainly due to binding of E_T. Therefore
$$\left|\frac{\Delta[X]}{X_T}\right| \sim \frac{k_1 E_T X_T}{X_T} \frac{1}{k_1 (X_T + K_m)} = \frac{E_T}{X_T + K_m} \ll 1. \tag{1.32}$$

The second condition is more restrictive and quasi-stationarity thus applies if $X_T \gg E_T$, as characteristic for most metabolic reactions, but typically not valid for protein signaling networks where both substrate and enzyme are typically proteins and $X_T \sim E_T$. Alternatively, if $X_T \leq E_T$ we can have $E_T, X_T \ll K_M$ such that the enzymatic reaction is limited by the formation of the complex relative to the processing rate (and C is therefore low, but almost constant) and the reaction then proceeds at speed $v \ll v_{max}$.

1.4.2 Hill Kinetics - Cooperativity

Many proteins have more than one binding site for their interaction partners (Fig. 1.1B (b) LHS). Binding of the first ligand can trigger a conformational change that alters the binding characteristics at all binding sites (Fig. 1.1B (b) 2nd column). The detailed modeling of all interactions and transitions is tedious. It can be shown [1] that if the first ligand binds with very low affinity (i.e. large $K_1 = \frac{k_{-1}+k_2}{k_1}$), and all subsequent ligands $i = 2...n$ binds with an increasing affinity (i.e. smaller K_i), then

$$\frac{d[P]}{dt} = v_{max} \frac{[X]^n}{K^n + [X]^n}. \tag{1.33}$$

Strictly speaking this formula is obtained in the limit $K_1 \to \infty$ and $K_n \to 0$ while keeping $K_1 K_n$ finite. $\frac{[X]^n}{K^n+[X]^n}$ is referred to as Hill function with Hill constant $K = (\prod_{i=1}^n K_i)^{\frac{1}{n}}$ and Hill coefficient n. If we plot the rate at which product is formed versus the substrate concentration we obtain a sigmoid graph (Fig. 1.1B (b) RHS). The Hill constant K corresponds to the concentration at which the reaction proceeds at half-maximal speed. The Hill factor n determines the steepness of the response. Typically n is smaller than the total number of binding sites because the idealized limits from above do not apply. For a more detailed discussion see standard text books in Mathematical Biology [2].

1.4.3 Inhibitory interactions

Inhibitors of a chemical reaction either fully prevent a reaction or reduce the reaction rate. When the effect of an inhibitor is reversible, the steady state of the inhibited species is reduced, whereas in the case of irreversible inhibition the steady state is zero. Here we will only focus on reversible inhibitions. An important regulatory paradigm is the use of inhibitors and activators to modulate the speed of reactions. Inhibitors can either compete with the substrate for the catalytic cleft (competitive inhibition) or alternatively inhibitors can induce a conformational change that alters the activity of the enzyme (allosteric inhibition).

Competitive Inhibition Inhibitors that bind to the active site of an enzyme and compete with substrate for access are termed competitive inhibitors (Fig.1.1B (c)). The set of differential equations which describes the system is (with C_2 referring to the CI complex):

$$\frac{d[X]}{dt} = k_{-1}[C_1] - k_1[E][X] \tag{1.34}$$
$$\frac{d[E]}{dt} = (k_{-1}+k_2)[C_1] - k_1[E][X] \tag{1.35}$$
$$\frac{d[I]}{dt} = -k_3[E][I] + k_{-3}[C_2] \tag{1.36}$$
$$\frac{d[C_1]}{dt} = -(k_{-1}+k_2)[C_1] + k_1[E][X] + k_{-3}[C_2] - k_3[E][I] \tag{1.37}$$
$$\frac{d[C_2]}{dt} = -k_{-3}[C_2] + k_3[E][I] \tag{1.38}$$
$$\frac{d[P]}{dt} = k_2[C_1] \tag{1.39}$$

We have $\frac{d[E]}{dt} + \frac{d[C_1]}{dt} + \frac{d[C_2]}{dt} = 0 \stackrel{\int}{\Rightarrow} [E] + [C_1] + [C_2] = E_T$. Using again a quasi steady-state approximation for C_1 and C_2 we have

$$C_1 = \frac{E_T[X]K_I}{[X]K_I + K_IK_m + [I]K_m} \tag{1.40}$$
$$C_2 = \frac{E_T[I]K_m}{[X]K_I + K_IK_m + [I]K_m} \tag{1.41}$$
$$\tag{1.42}$$

where $K_m = \frac{k_{-1}+k_2}{k_1}$ and $K_I = \frac{k_{-3}}{k_3}$. The product is then produced according to

$$\frac{d[P]}{dt} = k_2[C_1] = \frac{k_2E_T[X]K_I}{[X]K_I + K_IK_m + [I]K_m} = V_{max}\frac{[X]}{[X] + K_m(1+\frac{[I]}{K_I})} \tag{1.43}$$

A higher amount of substrate is therefore required to achieve a particular reaction rate: the half-saturation constant increases from K_m to $K_m(1+\frac{[I]}{K_I})$, where K_I is the dissociation constant for the enzyme-inhibitor interaction. Similarly, in case of Hill kinetics

competitive inhibition is modelled by an increase in the Hill constant K by a factor of $1+\frac{[I]}{K_I}$.

Allosteric Inhibition Allosteric inhibitors do not bind to the substrate binding site but affect the reaction rate by binding to a different site where they may induce a conformational change (Fig.1.1B (c)). While this conformational change can, in principle, also affect the binding affinities in the active site, allosteric inhibitors, in general, reduce the maximal velocity of the reaction v_{max} (i.e. $k_2' \ll k_2$ in Fig. 1.1B (c)), and we have

$$v = \frac{v_{max}}{1+\frac{[I]}{K_I}} \frac{[X]}{K_m + [X]}. \tag{1.44}$$

1.4.4 Goldbeter-Koshland Kinetics

The biological activity of signaling proteins is often controlled by a reversible chemical transformation, i.e. phosphorylation, methylation etc. If we were to model all steps explicitly the models would again be complex (Fig. 1.1B (d)), and experimental data may lack to parameterize the model. These enzymatic reactions are therefore often approximated with Michaelis-Menten reactions, i.e.

$$X + E_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} E_1 + X_p$$
$$X_p + E_2 \underset{p_{-1}}{\overset{p_1}{\rightleftharpoons}} C_2 \xrightarrow{p_2} E_2 + X.$$

We then have for the kinetics of the phosphorylated and unphosphorylated forms, X_p and X respectively,

$$\frac{d[X_p]}{dt} = -\frac{d[X]}{dt} = k_{phos} S \frac{X_T - [X_p]}{K_{M1} + X_T - [X_p]} - k_{dephos} \frac{[X_p]}{K_{M2} + [X_p]} \tag{1.45}$$

Here k_{phos} and k_{dephos} are the v_{max} of the enzymatic reactions. S refers to an external signal that is assumed to only affect the kinase and thus the phosphorylation reaction. In equilibrium

$$\frac{d[X_p]}{dt} = \frac{d[X]}{dt} = 0 \tag{1.46}$$

and we obtain the Goldbeter-Koshland formula

$$X_p^* = \frac{[X_p]}{X_T} = G(u_1, u_2, J_1, J_2) = \frac{2u_1 J_2}{B + \sqrt{B^2 - 4(u_2 - u_1)u_1 J_2}}. \tag{1.47}$$

where $u_1 = k_{phos}S$,$u_2 = k_{dephos}$,$J_1 = \frac{K_{M1}}{X_T}$,$J_2 = \frac{K_{M2}}{X_T}$, and $B = u_2 - u_1 + J_1 u_2 + J_2 u_1$. X_T refers to the total concentration of the signal protein X, i.e $X_T = [X] + [X_p]$. In the context of larger regulatory networks with such regulatory motif (Fig. 1.1B (d)), the Goldbeter-Koshland formula can be used to approximate the fraction of active enzyme dependent on the input signal S as long as quasi-stationarity for the reaction that regulates the enzyme relative to the rest of the network is a reasonable assumption.

Bibliography

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