Mathematical Modeling in Systems Biology

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Biological functionality arises from the complex interactions of simple components. Decades of intense research in molecular and cellular biology have led to detailed wiring diagrams for the regulatory interactions in many important signaling paths. Detailed biochemical and structural studies provide us with further information about the mode of interaction as well as about the kinetics of catalysed reactions. Biologists typically describe the complex interactions in signaling networks with cartoons (Fig. 1). The molecular species (genes, proteins, lipids, metabolites etc) and their complexes are depicted by icons; arrows indicate transformations, i.e. production, degradation, binding, dissociation, and chemical modifications (phosphorylation etc). For simple regulatory networks these cartoons are, in general, sufficient to grasp the regulatory logic of the circuit.

With the recent rise of high-thoughput technologies and the increased sophistication of computational tools an increasing regulatory complexity is being revealed with many more cross-interactions and interdependencies. As the wiring is getting more complex verbal reasoning quickly reaches its limitations in delineating the regulatory logic. Mathematical models are the method of choice to enable such an analysis.

The type of mathematical model depends on the particular setting. These notes will focus, in particular, on ordinary differential equation (ODE) models. These can be used when there are no spatial inhomogeneities, i.e. the components are "well-stirred", and the concentration of all components is sufficiently high such that a continuous description is applicable. If spatial inhomogeneities are important than partial differential equations (PDEs) need to used. If particle numbers are low stochastic effects are playing a role and the system has to be modelled with stochastic differential equations or with Monte Carlo methods.

Models cannot but approximate reality. The level of detail of a model is dictated by the question under investigation and by the type of data that is available to test the model with. Biological questions may concern qualitative properties of the regulatory system, i.e. its ability to show transient, sustained or oscillatory responses, its sensitivity to a given input, or its robustness to noise. A quantitative comparison of model predictions and experiments may be necessary to distinguish between competing hypotheses and to judge whether a certain regulatory behaviour is at all possible and plausible given the observed type and strengths of interactions and the speed of reactions.

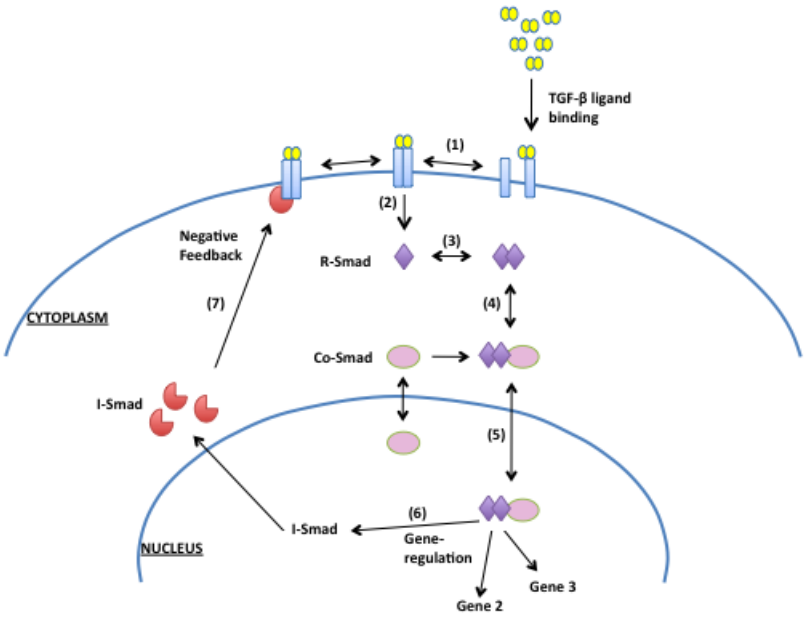

Figure 1: **A cartoon description of the TGF-beta signaling network.** TGF-beta is a soluble secreted protein, that signals by binding to the TGF-beta receptor (1). The ligand-bound receptor phosphoryates the regulatory Smad (R-Smad) (2). After dimerization (3), phosphorylated R-Smads bind a Co-Smad (4) and enter the nucleus (5) where they regulate a wide range of genes. One of the genes that is up-regulated encodes an inhibitory Smad (I-Smad) (6) that downregulates TGF-beta signaling by interfering with the receptor-dependent phosphorylation of the R-Smads (7).

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.

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 a differential equation of the form

$$\frac{dx_i}{dt} = \text{synthesis - degradation} \pm \text{shuttling} \pm \text{complex formation} \pm \text{chemical modification} \tag{1.1}$$

for all state variables $\{x_1(t), x_2(t), \ldots\}$. The values of all state variables at a given time point t constitute the state of the system at time t. The positive kinetic terms can be combined in a "gain" rate, the negative kinetic terms can be combined in a "loss" rate. 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

$$\frac{dx_i}{dt} = f(x_1, x_2, ..., x_n). \tag{1.2}$$

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.

1.1 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. 1.1. The law of mass action states that the rate of a reaction is proportional to the concentrations of the participating molecules. Based on this general approach we can distinguish the following basic reaction types that are frequently found in biological settings.

0th Order Reactions - Constant Reaction Rates

0th order reactions are the simplest of all reactions because the rate of the reaction is constant and does not depend on any time-varying species. 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, $[X]$,

$$\frac{d[X]}{dt} = k_{prod}. \tag{1.3}$$

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).

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.

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

$$\frac{d[X]}{dt} = -k_{deg}[X]. \tag{1.4}$$

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.

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 $[X_c]$. If X is exported from the nucleus at rate k_{out} and imported from the cytoplasm at rate k_{in} then we have

$$\begin{aligned} \frac{d[X_n]}{dt} &= k_{in}[X_c] - k_{out}[X_n] \\ \frac{d[X_c]}{dt} = -\frac{d[X_n]}{dt} &= -k_{in}[X_c] + k_{out}[X_n]. \end{aligned} \tag{1.5}$$

We can reformulate this set of equations by observing that the total amount of X is conserved, i.e. $[X_c]V_c + [X_n]V_n = T = const.$ V_c and V_n refer to the different volumes of cytoplasm and nucleus. These volume factors are important since only the number of proteins is conserved (according to conservation of mass). Given the difference in volume the concentrations are, however, not conserved. We can then write $[X_c] = \frac{T - [X_n]V_n}{V_c}$ and obtain a differential equation that is similar to Eq. 1.4 except for an additional constant term, $k_{in}\frac{T}{V_c}$,

$$\frac{d[X_n]}{dt} = k_{in}\frac{T - [X_n]V_n}{V_c} - k_{out}[X_n] = k_{in}\frac{T}{V_c} - (k_{in}\frac{V_n}{V_c} + k_{out})[X_n]. \tag{1.6}$$

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.

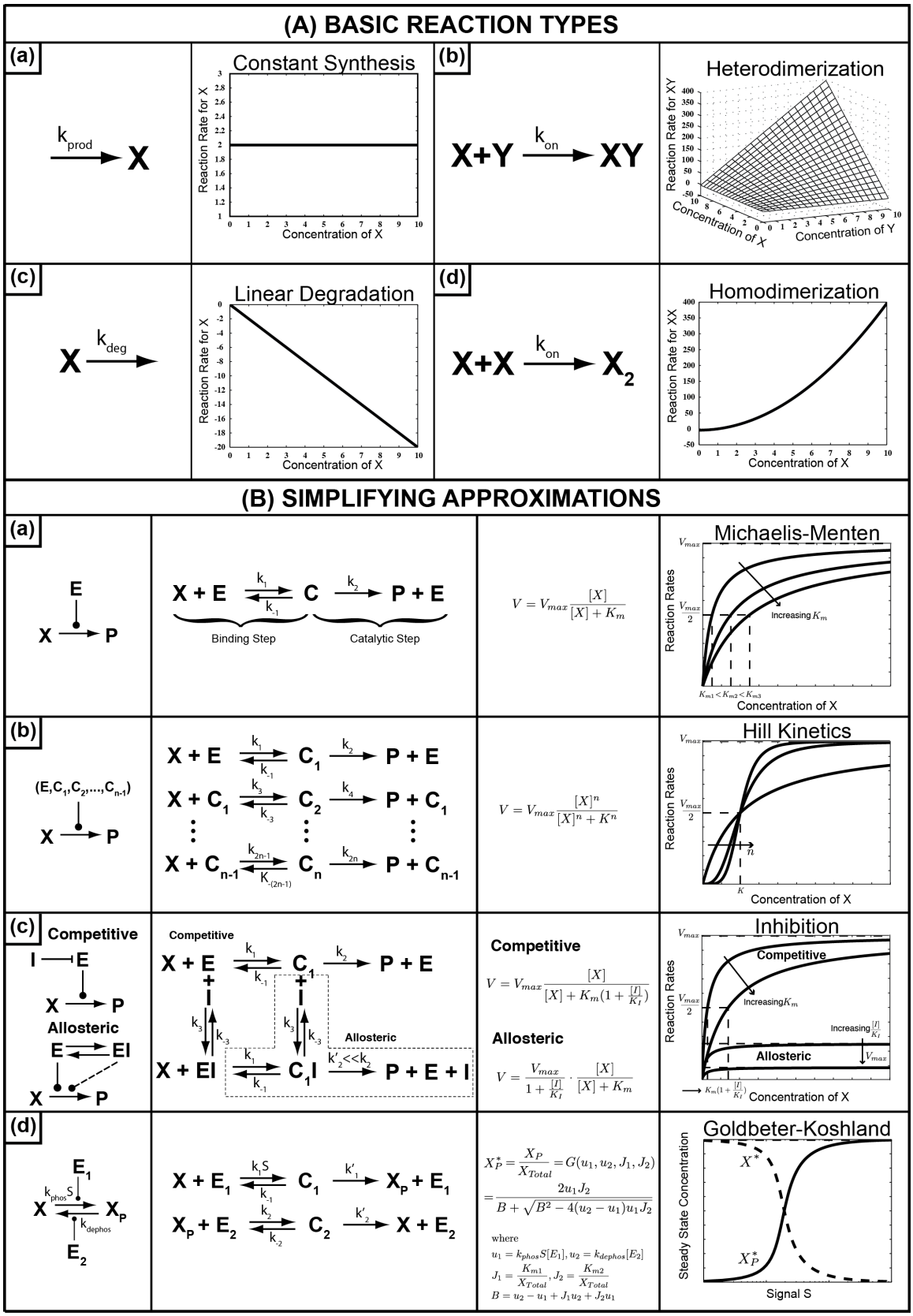

Figure 1.1: **Basic Reaction Types (A): (a)** Constant Synthesis. **(b)** Monomolecular Reactions: Linear Degradation. **(c)** Bimolecular Reactions: Heterodimer Formation. **(d)** Bimolecular Reactions: Homodimer Formation. **Simplifying approximations (B): (a)** Michaelis-Menten Kinetics. **(b)** Hill 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.

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

$$\frac{d[XY]}{dt} = k_{on}[X][Y] = k_{on}(X_T - [XY])(Y_T - [XY]) \tag{1.7}$$

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 $[X](t) = X_T - [XY(t)] = \frac{Y_T - X_T}{\frac{Y_T}{X_T}\exp((Y_T - X_T)kt) - 1}$.

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

$$\frac{d[X_2]}{dt} = k_{on}[X]^2 = k_{on}(X_T - 2[X_2])^2 \tag{1.8}$$

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 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.

1.2 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.

1.2.1 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,

$$X + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} E + P. \tag{1.9}$$

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

$$\frac{d[X]}{dt} = k_{-1}[C] - k_1[X][E] \tag{1.10}$$

$$\frac{d[E]}{dt} = (k_{-1} + k_2)[C] - k_1[E][X] \tag{1.11}$$

$$\frac{d[C]}{dt} = -(k_{-1} + k_2)[C] + k_1[E][X] \tag{1.12}$$

$$\frac{d[P]}{dt} = k_2[C] \tag{1.13}$$

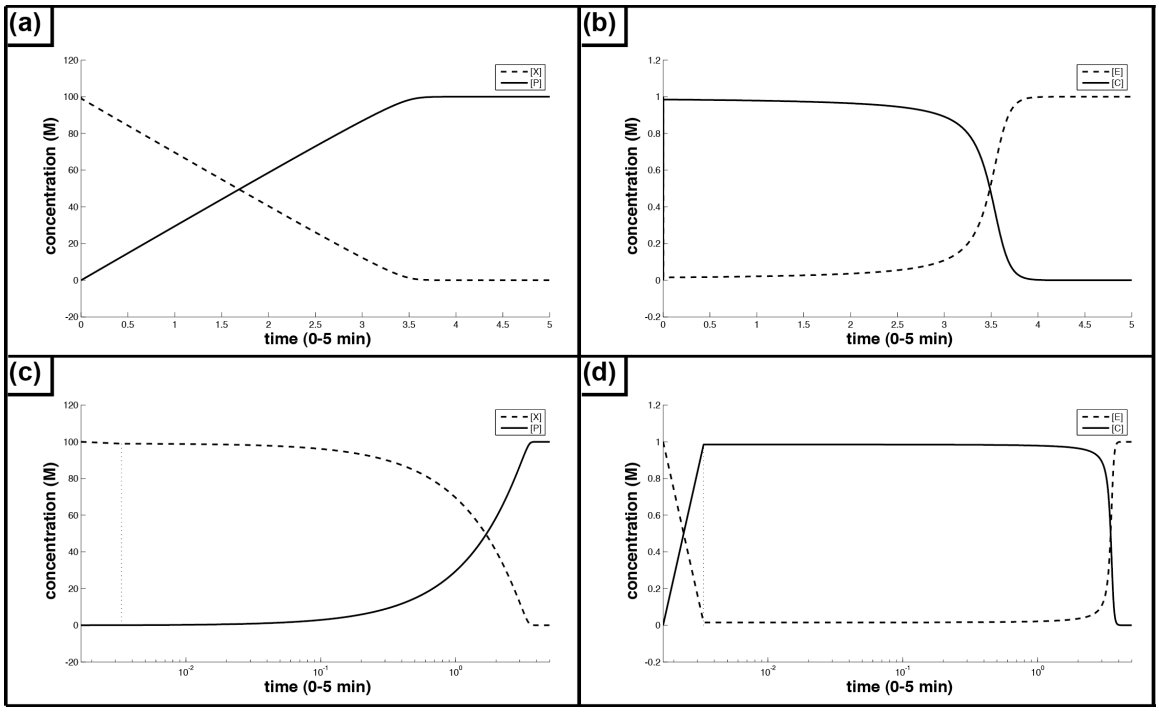

Figure 1.2: **The Kinetics of the Michaelis Menten Reaction (a, c)**The kinetics of substrate X and product P on linear and log scale. **(b,d)** The kinetics of enzyme E and substrate-enzyme complex C on linear and log scale.

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. 1.2B,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:

$$\frac{d[X]}{dt} = k_{-1}[C] - k_1[X](E_T - [C]) \tag{1.14}$$

$$\frac{d[C]}{dt} = -(k_{-1} + k_2)[C] + k_1(E_T - [C])[X] \tag{1.15}$$

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 $\tau = k_1 E_T t$, $\kappa_1 = \frac{k_{-1}+k_2}{k_1 S_T}$, and $\kappa_2 = \frac{k_{-1}}{k_1 S_T}$. (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, $[X]$, is much larger than the total enzyme concentration, $[E_T]$ and thus $\epsilon = \frac{E_T}{X_T} \ll 1$. We then obtain

$$\begin{aligned} \frac{ds}{d\tau} &= -s + c(s + \kappa_2) \\ \epsilon\frac{dc}{d\tau} &= s - c(s + \kappa_1) \end{aligned} \tag{1.16}$$

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

Quasi-steady-state approximation The quasi-state approximation $\epsilon \frac{dc}{d\tau} = 0$ yields as quasi-steady state $c = \frac{s}{s+\kappa_1}$. In dimensional form we then have for the rate at which the product (P) is formed the well-known Michaelis-Menten kinetics

$$\frac{d[P]}{dt} = k_2[C] = k_2 E_T \frac{k_1[X]}{k_1[X] + k_{-1} + k_2} = v_{max} \frac{[X]}{[X] + K_m}. \tag{1.17}$$

$k_2 E_T$ 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 ν can be approximated by $\nu = \nu_{max} \frac{[X]}{[X]+K_m}$.

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.

$$[C(t)] = [E_T] \frac{[X(t)]}{[X(t)] + K_m} \qquad \Rightarrow \qquad C(0) = 0 \neq E_T \frac{X_T}{X_T + K_m}. \tag{1.18}$$

There is thus an initial time interval in which C changes rapidly before assuming a relatively stable quasi-steady state value (Fig. 1.2C). 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 can be estimated from

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

$$\Leftrightarrow [C] = E_T \frac{X_T}{X_T + K_m} + \frac{1}{k_1(X_T + K_m)} \exp\left(-k_1(X_T + K_m)t\right). \tag{1.20}$$

as

$$T_c = \frac{1}{k_1(X_T + K_m)}. \tag{1.21}$$

The timescale on which X changes significantly can be estimated as

$$T_s = \frac{X_T}{|\frac{dX}{dt}|_{max}} \sim \frac{X_T + K_m}{k_2 E_T}. \tag{1.22}$$

Here we used the Michaelis-Menten formula for $d[X]/dt$; the reaction speed is maximal initially when $[X] \sim X_T$. We thus obtain the following constraints on the parameter values for quasi-stationairty to apply
(1) $T_c \ll T_s$ and thus $\frac{k_2 E_T}{k_1(X_T+K_m)^2} \ll 1$,
(2) In the time interval T_c, the substrate concentration is almost constant, i.e. $\frac{\Delta[X]}{X_T} \ll 1$ with $\Delta[X] \sim k_1 E_T X_T T_c$. Therefore

$$\frac{\Delta[X]}{X_T} \sim \frac{k_1 E_T X_T}{X_T} \frac{1}{k_1(X_T + K_m)} = \frac{E_T}{X_T + K_m} \ll 1. \tag{1.23}$$

We notice that the second condition is more restrictive. The quasi-stationarity thus requires that either $X_T \gg E_T$ as characteristic for most metabolic reactions, or $K_m >> X_T$ which would require that the enzyme is not saturated with substrate.

Perturbation Methods 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. 1.16, but perturbation methods can be used to obtain approximate solutions.

Regular perturbation methods
Regular perturbation methods can be used when the solution can be approximated arbitrarily close with a power series in a small parameter ϵ, i.e.

$$\begin{aligned} s(\tau,\epsilon) &= \sum_{n=0} \epsilon^n s_n(\tau) \\ c(\tau,\epsilon) &= \sum_{n=0} \epsilon^n c_n(\tau). \end{aligned} \tag{1.24}$$

Most often an acceptable approximation can be obtained by simply setting the small parameter ϵ to zero.

Inspection of Eqn.(1.16) shows that by setting $\epsilon\dot{c} = 0$ we reduced the order of the system of ODEs. As a result the initial conditions could no longer be matched. In case of such boundary value problems and multiscale problems singular perturbation methods need to be used.

Singular perturbation methods
There are a range of methods that can be used when a solution cannot be approximated by a single asymptotic expansion, including

- Method of matched asymptotic expansion
- WKB approximation
- Poincare-Linstedt method
- Method of multiple scales
- Periodic averaging

Method of matched asymptotic expansion From above we know that the solution differs close to $\tau = 0$ and for large τ. The thin layer of order $O(\epsilon)$ near $\tau = 0$ is sometimes called the boundary layer and is the τ-domain where there are very rapid changes in the solution. To proceed in a systematic singular perturbation way, we first look for the outer solution of the system which is valid for $\tau > 0$.

Upon substitution of Eqn.(1.24) into Eqn.(1.16) and equating powers of ϵ we obtain a sequence of differential equations for the $s_n(\tau)$, $c_n(\tau)$. The order 1, $O(1)$, equations are those without any ϵ, i.e.

$$\begin{aligned} \frac{ds_0}{d\tau} &= -s_0 + c_0(s_0 + \kappa_2) \\ 0 &= s_0 - c_0(s_0 + \kappa_1), \end{aligned} \tag{1.25}$$

and the $O(\epsilon)$ equations are those that are linear in ϵ, i.e.

$$\begin{aligned} \frac{ds_1}{d\tau} &= -s_1(c_0 - 1) + c_1(s_0 + \kappa_2) \\ \frac{dc_0}{d\tau} &= s_1(c_0 - 1) - c_1(s_0 + \kappa_1). \end{aligned} \tag{1.26}$$

We do not have initial conditions, and the solutions therefore involve undetermined constants of integration, one at each order, which have to be determined by matching these solutions as $\tau \to 0$ with those that we obtain from the inner solution. The inner solution can be determined by rescaling time so that we expand the boundary layer where c changes rapidly, i.e.

$$\sigma = \frac{\tau}{\epsilon}, \qquad s(\tau,\epsilon) = S(\sigma,\epsilon), \qquad c(\tau,\epsilon) = C(\sigma,\epsilon). \tag{1.27}$$

We then have

$$\begin{aligned} \frac{dS}{d\sigma} &= \epsilon(-S + C(S + \kappa_2)) \\ \frac{dC}{d\sigma} &= S - C(S + \kappa_1) \end{aligned} \tag{1.28}$$

The initial conditions are $S(0) = 1$, $C(0) = 0$. At $O(1)$ we have

$$\begin{aligned} \frac{dS_0}{d\sigma} &= 0 \\ \frac{dC_0}{d\sigma} &= S_0 - C_0(S_0 + \kappa_1) \end{aligned} \tag{1.29}$$

which is not of lower order than the original system, and can be solved to give the singular or inner solution

$$S_0(\sigma) = 1, \qquad C_0(\sigma) = \frac{1}{1+K}(1 - \exp(-1(1+K)\sigma) \tag{1.30}$$

which is valid for $0 \leq \tau \ll 1$. At $O(\epsilon)$ we have

$$\begin{aligned} \frac{dS_1}{d\sigma} &= -S_0 + C_0(S_0 + \kappa_2) \\ \frac{dC_1}{d\sigma} &= S_1(1 - C_0) - C_1(S_0 + \kappa_1). \end{aligned} \tag{1.31}$$

We now match the inner and the outer solutions by requiring

$$\lim_{\sigma\to\infty} S = 1 = \lim_{\tau\to 0} s, \qquad \lim_{\sigma\to\infty} C = \frac{1}{1+\kappa_1} = \lim_{\tau\to 0} c. \tag{1.32}$$

The initial conditions for the outer solutions are therefore $s(0) = 1$ and $c_0(0) = \frac{s_0(0)}{s_0(0)+\kappa_1} = \frac{1}{1+\kappa_1}$. In summary, we have as uniformally valid asymptotic solutions up to $O(\epsilon)$

$$\begin{aligned} s(\tau,\epsilon) &= s_0(\tau) + O(\epsilon) \\ c(\tau,\epsilon) &= C_0(\sigma) + O(\epsilon) \qquad \text{if} \quad 0 < \tau \ll 1 \\ &= c_0(\tau) + O(\epsilon) \qquad \text{if} \quad 0 < \epsilon \ll \tau \end{aligned} \tag{1.33}$$

with $s_0(\tau) + \kappa_1 \ln(s_0(\tau)) = 1 - (\kappa_1 - \kappa_2)\tau$, $C_0(\sigma) = \frac{1}{1+K}\left(1 - \exp\left(-(1+K)\frac{\tau}{\epsilon}\right)\right)$, $c_0(\tau) = \frac{s_0(\tau)}{s_0(\tau)+\kappa_1}$. We could now proceed to calculate higher order terms but the first order terms provide, in general, already a very good approximation, and the expressions for higher order terms become increasingly more complicated.

1.2.2 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 [2] 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

$$\frac{d[P]}{dt} = v_{max}\frac{[X]^n}{K^n + [X]^n}. \tag{1.34}$$

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 [3].

1.2.3 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):

$$\frac{d[X]}{dt} = k_{-1}[C_1] - k_1[E][X] \tag{1.35}$$
$$\frac{d[E]}{dt} = (k_{-1} + k_2)[C] - k_1[E][X] \tag{1.36}$$
$$\frac{d[I]}{dt} = -k_3[E][I] + k_{-3}[C_2] \tag{1.37}$$
$$\frac{d[C_1]}{dt} = (k_{-1} + k_2)[C_1] - k_1[E][X] + k_{-3}[C_2] - k_3[E][I] \tag{1.38}$$
$$\frac{d[C_2]}{dt} = -k_{-3}[C_2] + k_3[E][I] \tag{1.39}$$
$$\frac{d[P]}{dt} = k_2[C_1] \tag{1.40}$$

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

$$C_1 = \frac{E_T[X]K_I}{[X]K_I + K_IK_m + [I]K_m} \tag{1.41}$$
$$C_2 = \frac{E_T[I]K_m}{[X]K_I + K_IK_m + [I]K_m} \tag{1.42}$$
$$\tag{1.43}$$

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

$$\frac{d[P]}{dt} = k_2[C_1] = \frac{k_2E_T[X]K_I}{[X]K_I + K_IK_m + [I]K_m} = V_{max}\frac{[X]}{[X] + K_m(1 + \frac{[I]}{K_I})} \tag{1.44}$$

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

$$v = \frac{v_{max}}{1 + \frac{[I]}{K_I}}\frac{[X]}{K_m + [X]}. \tag{1.45}$$

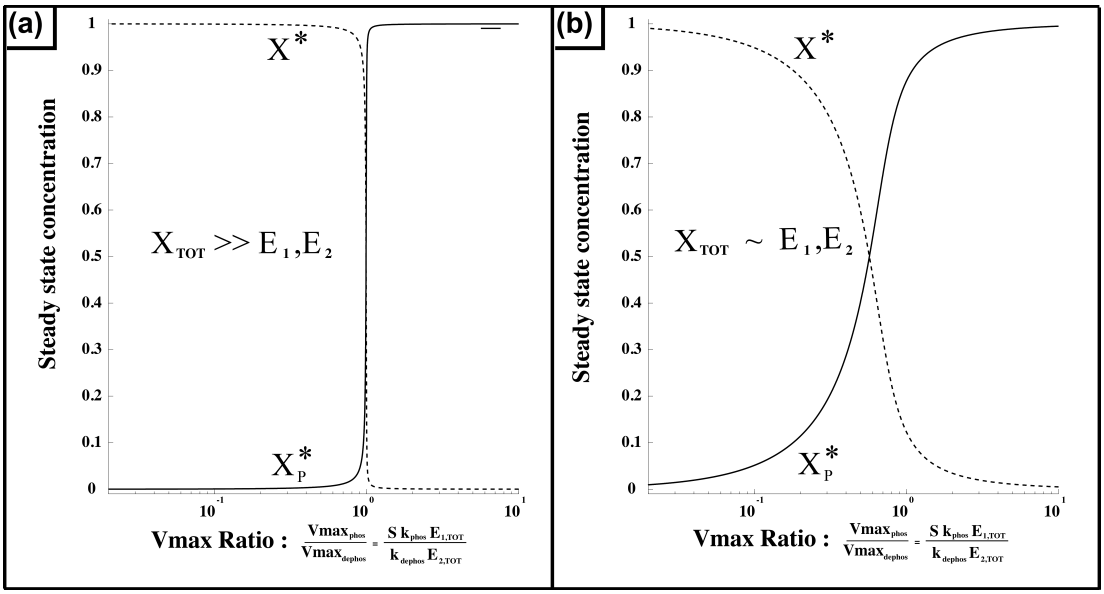

Figure 1.3: **Ultrasensitivity in the Goldbeter-Koshland Kinetics. (a)** If the enzymes are saturated with substrate ($X_T \gg E_T$) then a small change in v_{max} can greatly alter the response. **(b)** Without enzyme saturation the response is much less sensitive to changes in v_{max}.

1.2.4 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, and we have for the kinetics of the phosphorylated and unphosphorylated forms, X_p and X respectively,

$$\frac{d[X_p]}{dt} = -\frac{d[X]}{dt} = k_{phos} S \frac{X_T - [X_p]}{K_{M1} + X_T - [X_p]} - k_{dephos} \frac{[X_p]}{K_{M2} + [X_p]} \tag{1.46}$$

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

$$\frac{d[X_p]}{dt} = \frac{d[X]}{dt} = 0 \tag{1.47}$$

and we obtain the Goldbeter-Koshland formula

$$X_p^* = \frac{[X_p]}{X_T} = G(u_1, u_2, J_1, J_2) = \frac{2u_1 J_2}{B + \sqrt{B^2 - 4(u_2 - u_1)u_1 J_2}}. \tag{1.48}$$

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.

1.2.5 Ultrasensitivity

Reactions that respond with greater sensitivity to a signal X then expected from a Michaelis-Menten kinetics are termed ultrasensitive [1]. Ultrasensitivity is achieved in the Goldbeter-Koshland kinetics if the

Michaelis-Menten constants are small and the enzymes are saturated. In the limit we have zeroth-order kinetics and thus zeroth-order ultrasensitivity. A small change in v_{max} due to stimulation then leads to a sharp increase in the signaling component. However, as soon as the stimulation is removed the system switches back.

The Goldbeter-Koshland model describes the impact of two enzymes with opposing activity (e.g. kinase/phosphatase, methylase/demethylase etc) on a common substrate X that exists in two states, say X and X_p, by

$$\frac{d[X_p]}{dt} = -\frac{d[X]}{dt} = k_{phos} S \frac{X_T - [X_p]}{K_{M1} + X_T - [X_p]} - k_{dephos} \frac{[X_p]}{K_{M2} + [X_p]}. \tag{1.49}$$

A sensitive, switch-like response can be obtained when both enzymes are saturated (i.e. their Michaelis-Menten constants are low relative to the substrate concentrations (i.e. $K_{M2}/[X_p] \ll 1$, $K_{M1}/[X] \ll 1$) as is achieved by a high affinity of binding and a low maximal catalytic rate).

Suppose the common substrate, X, is mostly phosphorylated (i.e. $k_{dephos} < k_{phos}$). If both enzymes are saturated with substrate then increasing the activity of the enzyme with the lower v_{max} (here the phosphatase) will lead to an increased substrate concentration (unphosphorylated protein in this case) for the enzyme with the higher vmax (here the kinase). However, since the kinase is already saturated with substrate this will not enhance the rate at which proteins are phosphorylated. If the v_{max} of the phosphatase is sufficiently increased so that its v_{max} is now higher than that of the kinase then the proteins will switch from being mainly phosphorylated to being mainly unphosphorylated. Even a small increase in the phosphatase concentration can be sufficient to trigger such a switch if the v_{max} of the two enzymes are similar to start with. This then results in the observed ultrasensitivity to small changes in enzyme activity.

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