Chapter 8

Using Chemical Kinetics to Model Biochemical Pathways

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Abstract

Chemical kinetics is the study of the rate of reactions transforming some chemical entities into other chemical entities. Over the twentieth century it has become one of the cornerstones of biochemistry. When in the second half of the century basic knowledge of cellular processes became sufficient to understand quantitatively metabolic networks, chemical kinetics associated with systems theory led to the development of what would become an important branch of systems biology.

In this chapter we introduce basic concepts of chemical and enzyme kinetics, and show how the temporal evolution of a reaction system can be described by ordinary differential equations. Finally we present a method to apply this type of approach to model any regulatory network.

Key words Chemical kinetics, Chemical entities, Quantitative, Metabolic network, Systems biology, Enzyme kinetics, Regulatory network

1 Introduction to Chemical Kinetics

A living cell is built up as a series of compartments of various dimensions. The plasma membrane is an example of a bi-dimensional compartment surrounding the cytosol, which is itself a tridimensional compartment. Microtubules are examples of unidimensional compartments. These compartments can be considered both as containers—we can count the number of instances of a certain type of entity present in, or attached to, a compartment—and as diffusional landscapes—the movements of the entities within the compartment depend on its properties. Within the compartments, the entities can move and react with each other. The object of chemical kinetics is to study the temporal evolution of the positions and quantities of the entities contained in a compartment, sometimes called a reactor. In this chapter, we will not deal with the displacement of the chemical entities within a compartment. We will assume that an entity-pool, that is a set of entities that are indistinguishable as far as the model is concerned, is distributed homogeneously within the compartment. This hypothesis is known as the *well-stirred*

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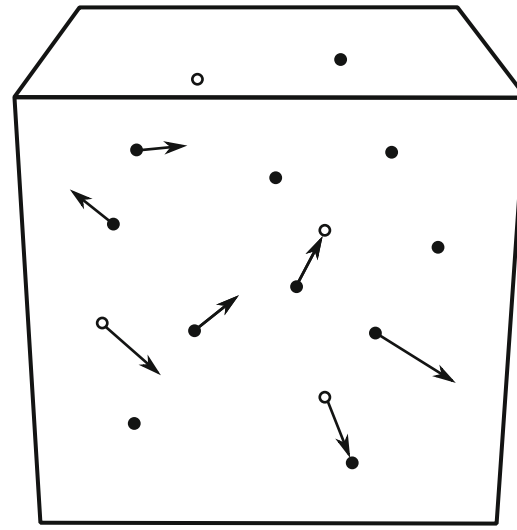

Fig. 1 Representation of a well-stirred container with two types of entities, represented by *empty* and *filled* circles. The *arrows* represent the direction and speed of their movements

approximation (Fig. 1). This approximation is based on the assumption that there is no diffusional anisotropy in the compartment, i.e., the molecules move randomly in any dimension. This is obviously a strong simplification in most of the cases pertaining to biological functions. It has nevertheless proved to be very useful in the past. In addition, the alternative requires to enter the realm of reaction–diffusion modeling, which involves not only more complex methods but also knowledge of distribution and diffusion characteristics of the reacting entities.

1.1 Chemical Reactions

A chemical reaction is the transformation of one set of substances called reactants into another set called products. At a microscopic scale, such a transformation is in general reversible, although there are many cases in which the reverse reaction is of negligible importance compared to the forward one. In all cases, a reversible reaction can be split into forward and reverse reactions. For a given reaction, reactants generally combine in discrete and fixed ratios to form products. These ratios indicate the amount of each substance involved in the reaction. The amounts consumed or produced in one reaction event are called the stoichiometric coefficients or numbers, ν_X, and are positive for products, and negative for reactants. If a substance is neither consumed nor produced by a reaction, its stoichiometric coefficient is 0. Equation 1 depicts a general reaction, in which A and B are reactants combining to form the product P. ν_A would be $-a$, $\nu_B = -b$ and $\nu_P = p$. The list $\{-a, -b, p\}$ is also called the stoichiometry of the reaction.

$$a\mathrm{A} + b\mathrm{B} \rightarrow p\mathrm{P} \tag{1}$$

In many cases in biology only an overall transformation consisting of many sequential reactions is experimentally observable. In the finest grained form these reactions are also known as elementary reactions. An elementary reaction is defined as a minimal irreversible reaction with no stable intermediary products. The lumped stoichiometric coefficients of the overall reaction consist of the sums of the stoichiometric coefficients for each reactant over all elementary reactions.

Chemical kinetics is concerned with the velocity of such transformations, the rates with which substances are consumed and produced. As the rate of change for a reagent depends on its stoichiometric coefficients, it can be different for individual substances. Therefore it is convenient to define the reaction rate, ν, as the rate of change of a substance divided by its stoichiometric coefficient. This effectively represents the number of reaction events taking place per unit of time and unit of compartment size.

$$\nu = \frac{1}{-a}\frac{\mathrm{d[A]}}{\mathrm{d}t} = \frac{1}{-b}\frac{\mathrm{d[B]}}{\mathrm{d}t} = \frac{1}{p}\frac{\mathrm{d[P]}}{\mathrm{d}t}$$

Therefore, we can compute the change of each substance as the product of the reaction rate and its stoichiometric coefficient for this reaction.

$$\frac{\mathrm{d[A]}}{\mathrm{d}t} = -a \times \nu$$
$$\frac{\mathrm{d[B]}}{\mathrm{d}t} = -b \times \nu$$
$$\frac{\mathrm{d[P]}}{\mathrm{d}t} = p \times \nu$$

Reaction rates depend on many factors and can effectively take any form for the purpose of modeling. In the following subsections, we will describe the simple cases where the reaction rates depend solely on the concentrations of the reacting substances.

1.2 Mass-Action Kinetics

For a chemical reaction to take place, the participants have to collide or come into close vicinity of each other. The probability of such collisions depends, among other parameters, on the local density of the reactants, and hence, in well-stirred environments, on their concentrations.[1] This relationship was first described by Guldberg and Waage in the second half of the nineteenth century in a series of articles on the dynamical nature of the chemical equilibrium [1]. They assumed that at equilibrium both the forward and

[1] Under nonideal conditions, as found in biology, activities instead of concentrations should actually be used both for describing rate equations and equilibria. As this is not common practice in biological modeling, we do not distinguish between activities and concentrations in the following. It should be noted, though, that activities can differ significantly from concentrations in cellular environments.

backward reaction forces or velocities were equal, and that these velocities where proportional to the concentrations of the reactants to the power of their stoichiometric coefficients. The relationship of reaction velocities and concentrations is called the "Law of Mass-Action", and rate expressions equivalent to the ones employed in their articles are sometimes referred to as "Mass-Action Kinetics".[2]

The rates of simple unidirectional chemical reactions are usually proportional to the product of the concentrations of the reactants to the power of constant exponents, called *partial reaction orders* or n_X. The sum of all partial orders is called the *order n* of a reaction and the proportionality factor is called the *rate constant k*. As the name indicates, this parameter does not vary in a given system. For example, for the reaction described in Eq. 1 assuming mass-action kinetics the reaction rate appears as follows:

$$v = k \times [A]^{n_A} \times [B]^{n_B}$$

The reaction has an order of $n = n_A + n_B$. In general, the order of elementary reactions is equal to the number of molecules interacting, also known as the *molecularity*. A *unimolecular* reaction $A \rightarrow P$ for example would have an order of one, a *bimolecular* reaction, such as $2A \rightarrow P$ or $A + B \rightarrow P$ would be a second-order reaction etc. However, this equivalence is not always true, and anisotropy or crowding of the reaction environments may affect the motion of molecules, resulting in different, and sometimes nonintegral, reaction orders.

While mass-action kinetics are strictly only valid for elementary reactions, they are widely and successfully applied in various fields of mathematical modeling in biology. Especially for large and vaguely defined reaction networks, as found in signal transduction, mass-action kinetics are commonly employed as a very general initial approach. Most often, the partial orders are taken to be identical to the stoichiometric coefficients. The rate constants can either be calculated from separately measured equilibrium constants and characteristic times, or computationally fitted to reproduce experimental results.

1.2.1 Zeroth Order Reactions

Reactions of order zero have a reaction rate that does not depend on any reactant. Zeroth order reactions can be used for instance to represent constant creations from boundary condition reactants, such as:

$$X \xrightarrow{k} P$$

[2]The term *mass-action* stems from the proportionality of the so-called reaction "force" to the mass of a substance in a fixed volume, which is proportional to the molar concentration of a substance.

where X represent a set of source reactants that are not depleted by the reaction. The reaction rate is then equal to:

$$v = k \times [\mathrm{X}]^0 = k$$

in which k is the rate constant, and has the units of a concentration per time.

1.2.2 First-Order Reactions

In general unimolecular reactions are modeled using first-order mechanisms. In irreversible first-order reactions, the reaction rate linearly depends on the concentration of the reactant. Many decay processes show such kinetics, for example radioactive decay, dissociation of complexes or denaturation of proteins. For a simple reaction:

$$\mathrm{A} \xrightarrow{k} \mathrm{P}$$

the following rate law applies:

$$v = k \times [\mathrm{A}]$$

in which k is the first-order rate constant, and has the units of a reciprocal time, [1/time]. If this is the only reaction affecting the concentration of A in a system, the change of [A] equals the negative reaction rate.Similarly, the change of [P] equals the reaction rate.

$$\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -v = -k[\mathrm{A}]$$
$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = +v = +k[\mathrm{A}]$$

1.2.3 Second-Order Reactions

Second-order reactions are often used to model bimolecular reactions, either between different types of molecules or between two instances of the same molecules. Examples are complex formation and dimerization reactions. For a simple reaction:

$$\mathrm{A} + \mathrm{B} \xrightarrow{k} \mathrm{P}$$

the following rate law applies:

$$v = k \times [\mathrm{A}] \times [\mathrm{B}]$$

in which k is the second-order rate constant, and has the unit of [1/(time × concentration)]. The change of [P] with time is described by the following differential equation:

$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = v = k \times [\mathrm{A}] \times [\mathrm{B}]$$

A special case of bimolecular reaction is when two reactant molecules of the same type react to form the product, for example in protein dimerization reactions. For the general reaction:

$$2\mathrm{A} \xrightarrow{k} \mathrm{P}$$

the reaction velocity and the temporal development of [A] and [P] are given by the following equations:

$$v = k \times [\mathrm{A}] \times [\mathrm{A}]$$
$$\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -2v = -2k[\mathrm{A}]^2$$
$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = v = k[\mathrm{A}]^2$$

Note that this formula is only valid because we assume a very large number of molecules are available to react. If picking one molecule changes significantly the probability to pick a second one, we must replace $[\mathrm{A}]^2$ by $[\mathrm{A}] \times (([\mathrm{A}]V - 1)/V)$, where V the volume of the reactor.

1.3 Representing the Evolution of Multi-Reaction Systems

In the sections above, we only derived expressions describing the temporal evolution of species altered by single reactions. In biological systems, substances are involved in many different processes, leading to complex ordinary differential equation systems, that normally can only be solved numerically and with help of computers. Having carefully designed the elementary processes composing the system, reconstructing the differential equations representing the evolution of the different substances is a systematic and easy procedure. We already saw in Subheading 1.2.2 that the reaction:

$$\mathrm{A} \xrightarrow{k} \mathrm{P}$$

Could be modeled by the system:

$$\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -1v = -1k[\mathrm{A}]$$
$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = +1v = +1k[\mathrm{A}]$$

If the reaction is reversible, such as:

$$\mathrm{A} \underset{k_\mathrm{r}}{\overset{k_\mathrm{f}}{\rightleftharpoons}} \mathrm{P}$$

then we can consider it as a combination of two irreversible reactions, the rates of which depend on [A] and [P]:

$$v_\mathrm{f} = k_\mathrm{f} \times [\mathrm{A}]$$
$$v_\mathrm{r} = k_r \times [\mathrm{P}]$$

The evolution of both substances therefore depends on the forward and reverse reaction rates. A is consumed by the forward reaction and produced by the reverse reaction. It is the other way around for P.

$$\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -1v_\mathrm{f} + 1v_\mathrm{r} = -1k_\mathrm{f}[\mathrm{A}] + 1k_\mathrm{r}[\mathrm{P}]$$
$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = +1v_\mathrm{f} - 1v_\mathrm{r} = +1k_\mathrm{f}[\mathrm{A}] - 1k_\mathrm{r}[\mathrm{P}]$$

To understand how to handle non-unity stoichiometric numbers, consider the following dimerization:

$$2\mathrm{A} \underset{k_\mathrm{r}}{\overset{k_\mathrm{f}}{\rightleftharpoons}} \mathrm{P}$$

The forward reaction will be modeled using second-order kinetics, and the rates will therefore be:

$$v_\mathrm{f} = k_\mathrm{f} \times [\mathrm{A}]^2$$
$$v_\mathrm{r} = k_\mathrm{r} \times [\mathrm{P}]$$

As above the evolution of both substances therefore depends on the forward and reverse reaction rates. But this time two molecules of A are consumed by each forward reaction and produced by each reverse reaction. Therefore:

$$\frac{\mathrm{d}[\mathrm{A}]}{\mathrm{d}t} = -2v_\mathrm{f} + 2v_\mathrm{r} = -2k_\mathrm{f}[\mathrm{A}]^2 + 2k_\mathrm{r}[\mathrm{P}]$$
$$\frac{\mathrm{d}[\mathrm{P}]}{\mathrm{d}t} = +1v_\mathrm{f} - 1v_\mathrm{r} = +1k_\mathrm{f}[\mathrm{A}]^2 - 1k_\mathrm{r}[\mathrm{P}]$$

This approach can then be extended, independently of the size of the system considered. An ODE system will contain (at most) one differential equation for each substance. This equation will contain components representing the involvement of the substance in the different reactions of the system. For the substance S_n, involved in a system containing r reactions, the differential equation takes the following form:

$$\frac{\mathrm{d}[\mathrm{S}_n]}{\mathrm{d}t} = \sum_{i=1}^{r} \nu_{ni} v_i$$

ν_{ni} denotes the stoichiometric coefficient of S_n in reaction i, v_i the rate of this reaction. The resulting ODE system can also be represented in matrix notation, by introducing the stoichiometric matrix, $\mathbf{N}$, and the reaction rate vector, $\mathbf{v}$. The stoichiometric matrix, $\mathbf{N}$, contains a row for each of the n species in the system, and a column for each of the r reactions. Its entries, N_{ij}, are the stoichiometric coefficients, of substance i in reaction j. $\mathbf{v}$ is a column vector with each element v_i indicating the rate of the ith reaction. Using the above, the change of the concentration vector S over time is described by:

$$\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} = \mathbf{N} \cdot \mathbf{v}$$

2 Numerical Integration of ODE Models

Once a set of differential equations has been determined, to describe the changes of the variables per unit of time, the behaviour of the system can be obtained by fixing initial conditions and

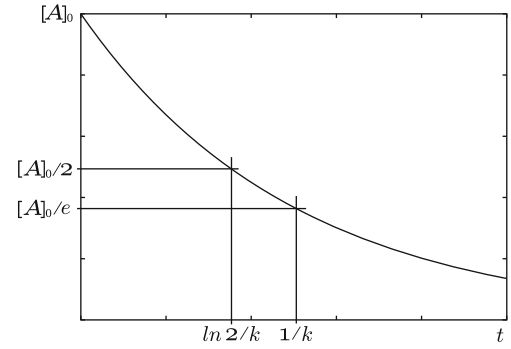

Fig. 2 Decay of a reactant A, that is consumed by a First-order reaction with a constant *k* from an initial concentration of $[A_0]$. The average lifetime of a given molecule of A, is given by $1/k$. [A] tends toward 0 while [P] tends towards $[A_0] + [P_0]$

solving the equations. In the case of a zeroth order reaction, the solution describing the evolution of P is of course a monotonic increase since a fixed amount of P is created per unit of time:

$$[\mathrm{P}](t) = [\mathrm{P}_0] + kt$$

The equation describing the evolution of A in a First-order reaction can be easily rearranged and analytically solved, assuming an initial concentration $[A_0]$ at time $t = 0$. Furthermore, since $[P]_t + [A]_t = [P_0] + [A_0]$:

$$[\mathrm{A}]_t = [\mathrm{A}_0] \times \mathrm{e}^{-kt}$$
$$[\mathrm{P}]_t = [\mathrm{P}_0] + [\mathrm{A}_0] \times (1 - \mathrm{e}^{-kt})$$

The rate constant in first-order kinetics is directly related to some characteristic times of substances, which are often readily available. For example the average life time of the reactant, τ, and the time it takes for its concentration to half, the half-life $t_{½}$, can be derived as (*see* Fig. 2):

$$\tau = \frac{1}{k}$$
$$t_{\frac{1}{2}} = \frac{\ln 2}{k}$$

Integration of the equation describing the evolution of P in a second-order reaction using the initial concentrations $[A_0]$, $[B_0]$ and $[P_0]$ leads to a hyperbolic time dependency:

$$[\mathrm{P}](t) = [\mathrm{P}_0] + [\mathrm{A}_0][\mathrm{B}_0]\frac{\mathrm{e}^{-kt[\mathrm{B}_0]} - \mathrm{e}^{-kt[\mathrm{A}_0]}}{[\mathrm{A}_0]\mathrm{e}^{-kt[\mathrm{B}_0]} - [\mathrm{B}_0]\mathrm{e}^{-kt[\mathrm{A}_0]}}$$

Contrarily from first-order reactions, the characteristic times in second-order reactions are not independent of the initial conditions, but depend on both the rate constant and the initial

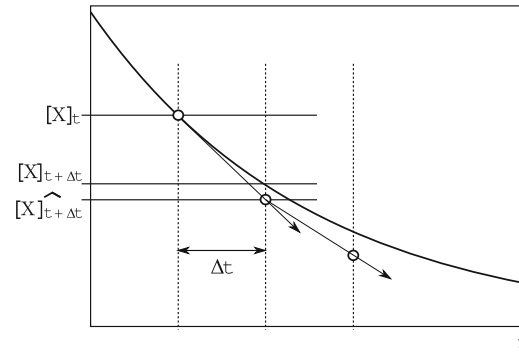

Fig. 3 Graphical representation of the forward Euler method to integrate ordinary differential equations. The *thick curve* represents [X] = *f*(*t*), and the vectors its derivative. Note the progressive error introduced by the coarse time discretization

concentrations of the reactants. In case the two reactants are instances of the same molecular pool (A = B), and assuming the initial concentrations to be $[A_0]$ and $[P_0]$, the resulting time courses for [A] and [P] are described by the following hyperbolic functions:

$$[A](t) = \frac{[A_0]}{2k[A_0]t + 1}$$

$$[P](t) = [P_0] + \frac{[A_0]^2 kt}{2k[A_0]t + 1}$$

However, beside the most elementary systems containing only few well-behaved reactions, we cannot generally solve a system of ordinary differential equations analytically. We have to resort to numerical integration, a method that goes back to the origin of differential calculus, where we approximate the current values of the variables based on the knowledge we have of their values in the (close) past. Many approximations have been developed. The simplest and easiest to grasp (but also the most error prone) is the forward Euler rule. If we discretize the time, one can make the following approximation:

$$\frac{d[X]}{dt} \approx \frac{\Delta[X]}{\Delta t} = \frac{\left([X]_{t+\Delta t} - [X]_t\right)}{\Delta t}$$

We can rearrange the equation above and extract the concentration as follows:

$$[X]_{t+\Delta t} \approx [X]_t + \frac{d[X]}{dt}(t) \times \Delta t$$

We know $d[X]/dt$ as a function of the vector of concentrations, obtained with the method described above, and can therefore compute the difference introduced during one Δt. This procedure is represented in Fig. 3. We can see on the figure that a systematic

error is introduced by the time discretization. Such an error becomes larger for more complex dynamics, such as non-monotonic behaviours (e.g., oscillations), or systems with fast and slow components. One can address the error by using tiny time steps but at the expense of computational efficacy. Many methods have been developed over the years to address this problem. A good introduction is given in LeMasson and Maex [2] and a more comprehensive survey of the field by Hairer et al. [3] and Hairer and Wanner [4]. Biological modeling tools such as COPASI [5], JDesigner/Jarnac [6], E-Cell [7] or CellDesigner [8] have their own in-built numerical ODE solver. They also generate the system of ODE to be solved automatically, so the required user input is limited to the list of chemical reactions in some defined format and of the parameters governing those reactions.

3 Modeling Biochemical Networks

Modeling the biochemical pathways does not require much more than what has been presented in Subheading 1. The only complexity we will introduce in the following sections are slightly more complex expressions for the reaction rates.

3.1 Basal Level and Homeostasis

Before modeling the effect of perturbations, such as extracellular signals, it is important to set up the right basal level for the substances that we will consider in the model. This basal level is obtained when the processes producing the substance and the processes consuming it are compensating each other. We then reach a steady state, where input and output are equal. To illustrate this, we will build the simplest system possible that permits to have a steady basal concentration of calcium. The system is made up of a continuous creation of calcium, for instance due to leaky channels in the plasma membrane or in the internal stores, modeled as a zero-order reaction (*see* Subheading 1.2.1). The calcium is then removed from the system for instance by pumps or buffers in excess, modeled as a first-order reaction (*see* Subheading 1.2.2).

$$\emptyset \xrightarrow{k_{in}} Ca^{2+} \xrightarrow{k_{out}} \emptyset$$

The instantaneous changes of calcium concentration then result from the combination of the two reaction rates (Fig. 4).

$$\frac{d[Ca^{2+}]}{dt} = k_{in} - k_{out}[Ca^{2+}]$$

The steady-state level is reached when the changes are null, that is $[Ca^{2+}] = k_{in}/k_{out}$. If the concentration of calcium is higher than this ratio, the second term wins and the concentration decreases. In contrast, if the concentration of calcium is lower than this ratio, the first term wins and the concentration increases. k_{out} can be

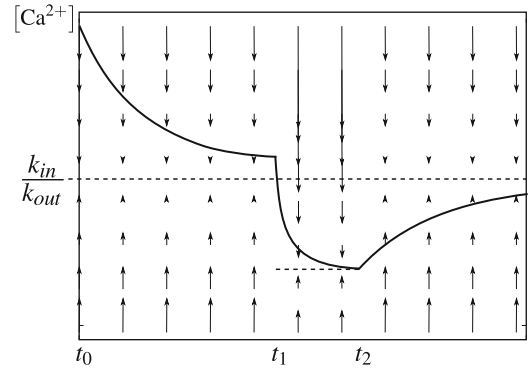

Fig. 4 Evolution of calcium concentration over time. Between t_0 and t_1, the extrusion is stronger than the creation. At t_1, k_{in} strongly decreases, for instance by a block of leak channels, and the concentration is brought to a lower steady-state value. At t_2 the block is removed. The creation becomes stronger than extrusion, and brings back the concentration to the initial steady state. *Vertical arrows* represent the intensity and direction of the reaction's flux for a given concentration of calcium

estimated from the decay observed after stimulation. k_{in} can therefore be computed from the steady state. Changing k_{in} in a discrete manner is a simple way of modeling the opening or closing of calcium channels. Such a homoeostatic control is extremely simple. More complex schemes can be designed, with control loops such as negative feed-backs on the creation steps and positive feed forwards on the extrusion steps.

3.2 Representing Enzymatic Reactions

In order to accelerate chemical reactions and select among different isomers, cells use enzymes, which are protein-based catalysts. They can increase reaction rates to a tremendous degree and often are essential to make reactions occur at a measurable rate. Enzyme catalyzed reactions tend to follow complex sequences of reaction steps, and the exact reaction mechanisms are generally unknown. The single reaction steps can be contracted into an overall description with lumped stoichiometries. However, since the detailed reaction mechanisms are most often unknown, and also parameters for each of these steps are hard to come by, such reactions can rarely be modeled considering each single step and using mass-action kinetics. Depending on how much detail is known, an enzyme catalyzed reaction can be described on different levels. The reaction equations for a simple conversion of a substrate S to a product P catalyzed by an enzyme E, for example, can vary depending on the consideration of intermediate enzyme complexes and reaction reversibility:

$$
\begin{aligned}
&S + E \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P\\
&S + E \rightleftharpoons ES \rightarrow EP \rightleftharpoons E + P\\
&S + E \rightleftharpoons ES \rightleftharpoons E + P\\
&S + E \rightleftharpoons ES \rightarrow E + P\\
&S + E \rightarrow E + P
\end{aligned}
\tag{2}
$$

Knowledge of the mechanism of an enzymatic reaction can be used to derive compact and simplified expressions fitting the overall kinetics. The alternative is to use generic rate laws that are known to loosely fit wide classes of reaction mechanisms, and to choose the ones that seem most appropriate for the reaction in question. The kinetics of the overall reaction are determined by the reaction mechanisms of the elementary steps, but exact derivations can become quite complex and cumbersome to handle. In general it is safer and more convenient to use approximate expressions in biological modeling, even more so as exact mechanisms are rarely known.

Two assumptions are available to simplify complex enzymatic reaction descriptions. The more general one is the quasi steady-state approximation, QSSA. The QSSA considers that some, or all, of the intermediary enzyme-substrate complexes tend to a near constant concentration shortly after the reaction starts. The other widely used assumption, called the rapid equilibrium assumption, is that some steps are much faster than the overall reaction, meaning that the participating enzyme forms are virtually at equilibrium and that their concentrations can be expressed using equilibrium constants. This approach is often used to model fast reactant or modulator binding to the enzyme. The application of these techniques depends very much on how much of the reaction mechanism is known. An excellent introduction into enzyme kinetics is given by Cornish-Bowden [9]. For a more exhaustive treatment with detailed derivations of rate laws for a multitude of mechanisms please refer to the standard work by Segel [10].

At the beginning of the twentieth century, Henri [11] proposed a reaction scheme and an accompanying expression for describing the rate of sucrose hydrolysis catalyzed by invertase. This reaction showed a deviation from normal second-order kinetics and tended to a maximal velocity directly proportional to the enzyme concentration. Making use of the existence of an intermediary substrate-enzyme complex, ES, and assuming that the substrate S and the enzyme E were in a rapid binding equilibrium with the complex, he could derive an expression fitting the experimental observations. A similar approach was taken and expanded in 1913 by Michaelis and Menten [12], who proposed the current form of the reaction rate based on a rapid equilibrium between enzyme and substrate.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

(k_2 is the catalytic constant, or turnover number, and often called k_{cat}). The QSSA was proposed as a more general derivation by Briggs and Haldane [13]. The substrate binding and dissociation, as well as the product formation step, lead to the following expression for the time dependence of [ES]:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

At steady state, the concentration of the intermediate complex, [ES], is constant hence $d[ES]/dt = 0$. Rearranging this equation and setting $K_M = \frac{k_{-1}+k_2}{k_1}$, we obtain $[E] = [ES] \times K_M/[S]$. Furthermore, because the concentration of enzyme is constant, we have $[E] = [E_t] - [ES]$. Equating both, we obtain:

$$v = \frac{d[P]}{dt} = k_2[ES] = k_2[E_t]\frac{[S]}{K_M + [S]} \tag{3}$$

$k_2 \times [E_t]$ is sometimes called the maximal velocity ν_{max}. This rate expression is often used—and abused—when modeling biochemical processes for which the exact mechanisms are unknown. However, one has to realize that it only holds true if the concentration of the enzyme-substrate complex stays constant, which in turns implies that the concentration of substrate is in large excess. Those conditions are very rarely met in signal transduction systems, resulting in many artifacts.

Plotting the reaction velocity, ν, against the substrate concentration, [S], gives a rectangular hyperbolic curve (*see* Fig. 5). The parameter K_M has the unit of a concentration and is of central importance in describing the form of the substrate dependence of the reaction velocity. As can be seen by inserting K_M for [S] in Eq. 3, it denotes the substrate concentration at which the reaction speed is half of the limiting velocity. If $[S] \ll K_M$, then [S] in the denominator can be disregarded and the reaction becomes linear with regard to S, showing first-order characteristics:

$$[S] \ll K_M \Rightarrow v \approx \frac{v_{max}}{K_M} \times [S]$$

On the other extreme, for high substrate concentrations, $[S] \gg K_M$, the reaction speed becomes virtually independent of [S] and tends toward ν_{max}.

$$[S] \gg K_M \Rightarrow v \approx v_{max} = k_{cat} \times [E_t]$$

Most enzyme catalyzed reactions show a similar rate behaviour inasmuch as they exhibit first or higher order dependencies on the substrate at lower substrate concentrations and tend to a limiting rate depending only on the enzyme concentration when the reactant concentrations are high.

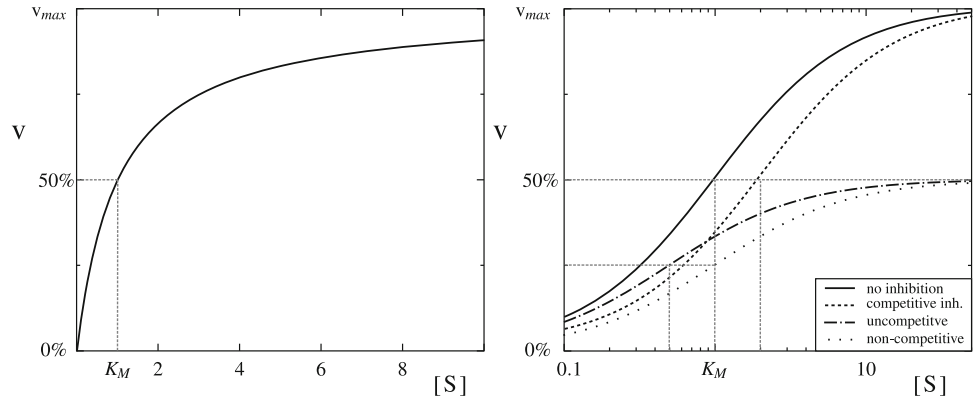

Fig. 5 Dependence of the reaction velocity, ν, of the irreversible Michaelis–Menten equation on the concentration of the substrate, S. The *left graph* shows the uninhibited case. On the *right* various forms of inhibition are shown in a semi-logarithmic plot. The *horizontal dotted lines* indicate the apparent half maximal velocities, the *vertical ones* the apparent K_Ms. Competitive inhibition does not alter the maximal velocity, but shifts the K_M to higher values, while non-competitive inhibition simply decreases the apparent V_{max}. The special case of uncompetitive inhibition leads to an apparent increase of substrate affinity of the enzyme, that is a lower K_M, but a reduction of the apparent V_{max}. Mechanistically this is due to the unproductive enzyme-substrate-inhibitor complex ($K_M = 1$; [I] $= 1$; comp., uncomp. and non-comp. inhib.: $K_i = 1$.)

While the original Michaelis–Menten equation was derived to describe the initial velocity of the enzymatic reaction in absence of product, allowing the reverse reaction to be neglected, the QSSA can also be used to derive a reversible Michaelis–Menten equation describing the most extensive reaction scheme in Eq. 2. Using the same procedure as above, the following expression for the reaction velocity in dependence of E_T, S and P can be derived:

$$v = \frac{v_{\text{fwd}} \frac{[\text{S}]}{K_{\text{MS}}} - v_{\text{rev}} \frac{[\text{P}]}{K_{\text{MP}}}}{1 + \frac{[\text{S}]}{K_{\text{MS}}} + \frac{[\text{P}]}{K_{\text{MP}}}} \tag{4}$$

As the net rate of a reversible reaction has to vanish at equilibrium, one of the parameters of Eq. 4 can be expressed using the equilibrium constant by setting the numerator of the expression to zero. The so called Haldane relationship connects kinetic and thermodynamic parameters of an enzymatic reaction. While some mechanisms lead to much more complicated expressions, at least one Haldane relationship exists for every reversible reaction.

$$K_{\text{eq}} = \frac{v_{\text{fwd}} K_{\text{MP}}}{v_{\text{rev}} K_{\text{MS}}} = \frac{k_2 K_{\text{MP}}}{k_{-1} K_{\text{MS}}}$$

3.3 Modeling Simple Transport Processes

Compartmentalization of molecular species and transport across membranes are of great importance in biological systems, and often need to be implicitly accounted for or explicitly included into models.

Transport across membranes can either occur passively by simple diffusion, or be coupled to another reaction to actively move molecules against a chemical potential gradient. In the simplest form of passive diffusion, molecules just directly pass through a membrane or an open channel or pore. As the connected compartments in general have differing volumes, the change of concentration of a substance flowing from one compartment to another is not equal in both compartments. Therefore the rate of translocation is commonly described by the flux, j, of a substance, that is the amount of a substance crossing a unit area per time unit. In case of no other influences on the translocation, but simple diffusion, the flux of a substance S into a cell through a membrane follows a variant of Fick's first law:

$$[S_{out}] \rightleftharpoons [S_{in}]$$
$$js = p_s([S_{out}] - [S_{in}])$$

in which $[S_{out}]$ and $[S_{in}]$ are the concentrations of S on the exterior and inside the cell, respectively. p_S denotes the permeability of the membrane for S. The permeability for direct diffusion is proportional to the diffusion coefficient of S and, for pores or channels, to the number of open channels per area.

To derive an expression of the change of concentration of S, it is important to consider that the flux is given as amount per area and time and not as concentration per time. Therefore the volumes of the exterior and the cell have to be included in the differential expressions of concentrations. The overall rate of translocation, ν_t, depends on the surface area, A, of the membrane, and the permeability and area can be contracted to a transport rate constant, $k_S = p_S \times A$. For the change of $[S_{out}]$ and $[S_{in}]$, respectively, the following expressions can be derived:

$$\frac{d[S_{out}]}{dt} = -\frac{v_T}{V_{out}} = -\frac{k_S}{V_{out}}([S_{out}] - [S_{in}])$$
$$\frac{d[S_{in}]}{dt} = \frac{v_T}{V_{in}}$$

with V_{out} and V_{in} being the volumes of the exterior and the cell.

In the case of a molecule that does not simply diffuse through a membrane or pore, but needs to bind a carrier to be translocated from one compartment to the other, the kinetic expressions depend on the exact mechanism of translocation. The simplest case of facilitated, or carrier-mediated, diffusion consists of a carrier with a single binding site, C, which can bind a substance A with equal affinity on each side of the membrane, and flips from one side of the membrane to the other. Using the steady-state approach the following expression can be derived for the translocation rate:

$$v_t = \frac{v_{max}([A_{out}] - [A_{in}])}{K_M + [A_{out}] + [A_{in}] + \frac{K_i[A_{out}][A_{in}]}{K_M}}$$

In this equation ν_{max} is the limiting rate of translocation and depends mostly on the amount of carrier. K_M is the concentration of A on one side at half maximal translocation in case of zero concentration on the other side of the membrane, and K_i, called the interactive constant, depends on the relative mobility of the free and loaded carrier (for details *see* Ref [14]).

4 Modeling Modulation of Dynamical Processes

Reactions in biological systems are not only regulated by the availability of reactants and catalysts, but also by compounds modulating the activity of channels and enzymes, often without any direct involvement in the specific reactions. Examples are neurotransmitters that alter the flow of ions through channels, without direct involvement in the transport process, enzyme allosteric effectors, that will modulate the activity of an enzyme without being involved in the catalytic reaction etc. In this section, we will introduce a generic method to model activation and inhibition of reactions, based on Hill equations.

4.1 Binding of Modulators and Activity

The activities of receptors, channels and enzymes are often regulated by ligands binding to them. One important characteristic of such binding processes is the *fractional occupancy*, $\overline{Y}$ of the bound compound. It is defined as the number of binding sites occupied by a ligand, divided by the total number of binding sites. For a ligand X binding to a single binding site of a protein P, we can express [PX] and $\bar{Y}$ as follows, using the dissociation constant $K_d = \frac{k_{off}}{k_{on}}$ and the total protein concentration $[P_T] = [P] + [PX]$:

$$\begin{gathered} P + X \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PX \\ [PX] = \frac{[P_T][X]}{K_d + [X]} \\ \bar{Y} = \frac{[PX]}{[P_T]} = \frac{[X]}{K_d + [X]} \end{gathered} \tag{5}$$

Equation 5, also known as the Hill–Langmuir equation, is very similar to the Michaelis–Menten equation. Like [S] in Eq. 3, [X] stands for the concentration of *free* ligand, but can be substituted with the total ligand concentration $[X_T] = [X] + [PX]$ in case that $[X_T] \gg [P_T]$. If P is active only when bound to X, one must multiply the reaction rate by $\bar{Y}$ to describe the actual reaction velocity. On the contrary, if P is active only when not bound to X, one must multiply the reaction rate by $1 - \bar{Y}$:

$$1 - \bar{\Upsilon} = 1 - \frac{[\mathrm{PX}]}{[\mathrm{P_T}]} = \frac{K_\mathrm{d}}{K_\mathrm{d} + [\mathrm{X}]} \tag{6}$$

A general form of Eq. 5 was developed by Hill [15]. Drawing on observations of oxygen binding to haemoglobin, Hill suggested the following formula for the fractional occupancy $\bar{\Upsilon}$ of a protein with several activator binding sites:

$$\bar{\Upsilon} = \frac{\frac{[\mathrm{X}]^h}{K_H}}{1 + \frac{[\mathrm{X}]^\mathrm{h}}{K_H}} = \frac{[\mathrm{X}]^h}{K_H + [\mathrm{X}]^h}$$

where [X] denotes ligand concentration, K_H is an apparent dissociation constant (with the unit of a concentration to the power of h) and h is the *Hill coefficient*, which needs not be an integer. The *Hill coefficient* h indicates the degree of cooperativity, and in general is different from the number of ligand binding sites, n. The Hill equation can show positive and negative cooperativity, for exponent values of $h > 1$ and $0 < h < 1$, respectively. In case of $h = 1$ it shows hyperbolic binding behaviour. With increasing exponents, the ligand binding curve becomes more and more sigmoid, with the limit of a step function with a threshold value of $\sqrt[h]{K_H}$. The constant $K_h = \sqrt[h]{K_H}$ provides the ligand concentration at which half the binding sites are occupied (equivalent to a dissociation constant), or, in purely phenomenological uses, activation or inhibition by the effector is half maximal. Note that negative values of h produce the same decreasing sigmoid function than the above $1 - \bar{\Upsilon}$, so the generalized Hill function can be used for both activators and inhibitors.

4.2 Modeling Regulation of Processes with Hill Functions

The Hill equation can easily be adapted to provide functions to describe interactions with little prior knowledge. Let us assume a gene which expression is regulated in a nonlinear fashion for instance by the binding of a transcription factor A. One can model the gene expression with increasing concentrations of A using a Hill function:

$$v = v_\mathrm{max} \times \frac{[\mathrm{A}]^h}{K_\mathrm{A}^h + [\mathrm{A}]^h} \tag{7}$$

Here ν is the actual production of mRNA by the gene. ν_max indicates the maximal activity of the gene. K_A and h indicate the transcription factor concentration for half maximal activation, and a cooperativity coefficient. If $[\mathrm{A}] = 0$, the correcting factor is close to 0, ν is null, i.e., there is no gene expression. If [A] is large, the correction factor is close to 1 and the expression is maximal. Similarly, the effect of a repressor I can be described by:

$$v = v_{\max} \times \frac{K_I^h}{K_I^h + [I]^h} \tag{8}$$

In this equation K_I stands for the transcription factor concentration triggering half maximal inhibition. If $[I] = 0$, the correcting factor is close to 1 the gene expression is maximal, while if [I] is large, the correction factor is close to 0 and so is the gene expression. Note that the same result is obtained with the mathematical expression derived for activation above, with exponents of $-h$. Therefore, one can provide a generic formula that can phenomenologically describe the effects of all independent activators and inhibitors at once.

$$v = v_{\max} \times \prod_{i=1}^{n} \frac{[X_i]^{h_i}}{K_{X_i}^{h_i} + [X_i]^{h_i}} \tag{9}$$

Such a formula can then be used in parameter estimation procedures. For n effectors, one has to estimate $2n$ independent parameters, or only n if the cooperativity is assumed negligible. Note that such a formula is only valid if no significant interactions take place between the effectors.

As an example of Hill equation use, let's study the kinetics of calcium-gated channels. An example containing two different types of activation is given in Borghans et al. [16] for the Ca^{2+} induced Ca^{2+} release (CICR) via the inositol triphosphate (InsP3) receptor. Equation 18 of the paper describes the release of calcium from a calcium sensitive pool. The flux rate is given by:

$$v_{\text{InsP3 R}} = v_{\max} \frac{[Ca_p]^2}{K_1^2 + [Ca_p]^2} \frac{[Ca_c]^2}{K_2^2 + [Ca_c]^2}$$

In this equation $\nu_{\max}$ denotes the maximal release rate, and $[Ca_p]$ and $[Ca_c]$ the Ca^{2+} concentrations in the pool and the cytoplasm. The release is regulated by the Ca^{2+} concentrations on both sides of the membrane separating the pool and the cytosol, and K_1 and K_2 stand for the threshold concentrations for these activations. Parthimos et al. [17] used an even more complex expression for the CICR from the sarcoplasmic reticulum via the InsP3 receptor. The receptor was modeled to be both activated and inactivated by cytosolic Ca^{2+}, Ca_c, using two Hill functions involving Ca_c. A possible mechanistic explanation for this form would be the existence of independent activation and inhibition sites, with different affinities and degrees of cooperativity for Ca^{2+}. In the flux rate through the InsP3 receptor

$$v_{\text{InsP3R}} = v_{\max} \frac{[Ca_s]^2}{K_1^2 + [Ca_s]^2} \frac{[Ca_c]^4}{K_2^4 + [Ca_c]^4} \frac{K_3^4}{K_3^4 + [Ca_c]^4} \tag{10}$$

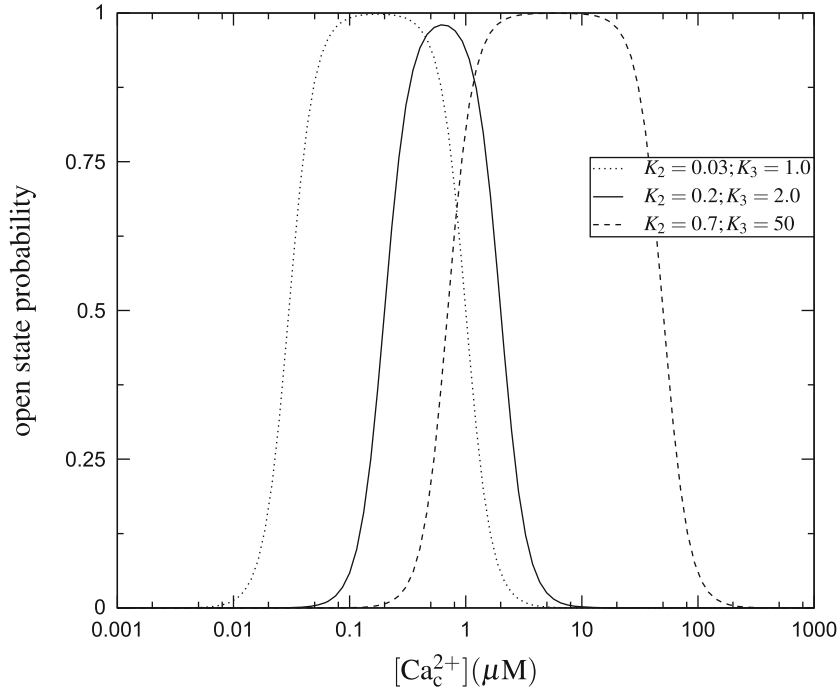

Fig. 6 InsP3 receptor opening probability dependent on cytoplasmic Ca^{2+} after Parthimos et al. [17] as described in Eq. 10. K_2 and K_3 indicate the concentrations of half maximal activation and inhibition, respectively, of the InsP3 receptor. For both activation and inhibition a Hill factor of 4 was assumed

K_2 and K_3 indicate the cytosolic Ca^{2+} concentrations at which activation and inhibition of CICR, respectively, are half maximal. If they are chosen in such a way that $K_2 < K_3$, the flux rate through the receptor reaches a maximum for concentration values between the values of the two constants and vanishes for higher cytosolic Ca^{2+} concentrations (*see* Fig. 6), creating a complex on–off behaviour of the InsP3 receptor in dependence of the Ca^{2+} concentration. In case of nonessential activation or leaky inhibition, a process can still proceed at a basal rate ν_{bas} in absence of the activator or at high concentrations of the inhibitor (Fig. 7). This can be accounted for by using the relative basal rate, $b = \frac{\nu_{bas}}{\nu_{max}}$.

$$v = v_{max}(b + (1 - b)\gamma([X]))$$

where $\gamma([X])$ is the function describing the relative activity in dependence of the concentration of the regulating agent X, that is $\bar{\Upsilon}$ or $1 - \bar{\Upsilon}$ mentioned in Eqs. 5 and 6. Note that if there is no reaction in the absence of a modulator, the basal rate is 0, $b = 0$, and the equation is equivalent to Eqs. 7 and 8. One can therefore further generalise Eq. 9 as:

$$v = v_{max} \times \prod_{i=1}^{n} \left(b_i + (1 - b_i) \times \frac{[X_i]^{h_i}}{K_{Xi}^{h_i} + [X_i]^{h_i}} \right)$$

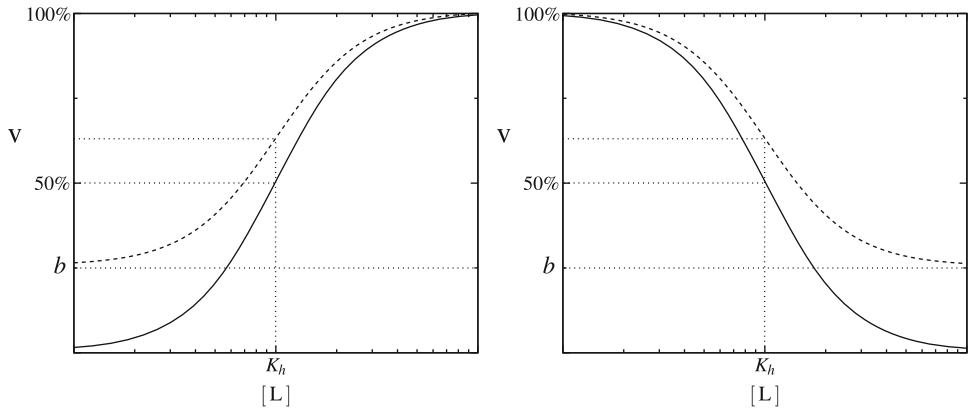

Fig. 7 Activation (*left*) and inhibition (*right*) modeled using Hill functions with a $n_h = 2$. Ligand concentration is shown in units of the concentration of half maximal activation or inhibition, respectively, K_h on a logarithmic scale and the velocity ν in percent of the fully activated or uninhibited velocity, ν_{max}. The *dashed line* shows cases with a basal rate, ν_{bas}, of 25 % of ν_{max} $\left(b = \frac{\nu_{\text{bas}}}{\nu_{\text{max}}} = 0.25\right)$

This equation provides an initial framework to model the kinetics of almost any regulatory network in the absence of mechanistic knowledge. Although Hill functions have been frequently used because of their simplicity, other generic frameworks have been proposed to phenomenologically model kinetics of regulatory networks, including logoid function [18], Goldbeter-Koshland switches [19], S-systems [20] etc. Further information can be found in a quite famous review [21]. As mentioned above, it is important to realize that such a framework assumes independence of the modulators. Other more complicated formulae, derived for enzyme regulation, can then be used if the concentration of a modulator affects the effect of another one. The reader should refer to Segel [10] for more details.

5 Further Reading

Biophysical chemistry, James P. Allen. This is a complete and concise presentation of the physical and chemical basis of life [22].

Computational Cell Biology, Christopher P. Fall, Eric S. Marland, John M. Wagner, John J. Tyson. Also known as "the yellow book", this is an excellent introduction to modeling cellular processes. It contains chapters dedicated to ion channels, transporter, biochemical oscillations, molecular motors and more [23].

Enzyme kinetics, Irwin H. Segel and Fundamentals of Enzyme Kinetics, Athel Cornish-Bowden. Also known as "the black book" and the "the red book", these are the two reference books if one wants to know how to model an enzymatic reaction, regardless of its complexity.

Solving Ordinary Differential Equations I and II, Ernst Hairer, Syvert P. Norsett, Gerhard Wanner.Extensive coverage of the domain of ordinary differential equations, from Newton and Leibniz to the most advanced techniques implicit solvers.

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