1 Introduction to Kinetics

The final aim of kinetic research is to obtain knowledge of the nature of the reaction from a study of its progress.

Michaelis and Menten (1913)[153]

In the new enzymology, mechanisms are defined by rigorous, comprehensive kinetic analysis based on direct measurement of reactions occurring at the active sites of enzymes. The field of enzymology has shown renewed growth due to excitement over the discoveries of new enzyme activities, greater understanding of the evolution of enzyme catalysis through enzyme families and superfamilies, and the application of this knowledge to engineer new functions. This has been coupled with enhancements in techniques for studying enzyme kinetics and structure, and the refinement of advanced molecular dynamics simulations to link events occurring at the molecular level to observable events. The rationale and techniques described in this book complement these studies by providing methods for definitive kinetic analysis to resolve reaction pathways.

Enzymes are dynamic, and a given crystal structure is only an approximation of reality in that it provides just one static view, which is influenced by the conditions required to obtain crystals. Many questions are left unanswered. What are the roles of enzyme conformational dynamics in enzyme activity and specificity? Are changes in structure coupled throughout the protein? Does the substrate bind before or after the enzyme transitions from an open to a closed state? How does the alignment of catalytic residues bring about a chemical reaction? These are important mechanistic questions that require accurate kinetic analysis.

In this text, we strive to develop an intuitive and quantitative understanding of reaction kinetics, using a combination of equation-based analysis and computer simulation to see patterns in data that reflect the underlying mechanism. By identifying these patterns, we can develop a testable model and then fit data using numerical integration of the rate equations to provide the most rigorous and comprehensive analysis possible without simplifying approximations. We begin with simple reaction kinetics and progress to more complex systems. Principles and intuition developed in learning to understand the dynamics of simple systems are then applied to aid in examining more complete, biologically or chemically relevant models.

We use the term "computer simulation" as shorthand to mean numerical integration of rate equations, which is distinguished from analytical integration to derive equations. Analytical integration requires approximations to solve the math, while numerical integration does not.

Kinetic analysis based on mathematical descriptions can appear rather abstract, and courses dedicated to teaching kinetics often get bogged down on math, consuming time that could be dedicated to teaching principles

Facing page: Sunrise over Rio de Janeiro. This picture symbolizes the dawn of the new enzymology

of kinetics. Therefore, throughout the text, we illustrate kinetic concepts with tutorials using computer simulation to provide a visual link between experimental observations, a proposed model, and a set of rate constants and starting conditions. Patterns in data that suggest a model are also revealed by traditional equation-based data fitting, but it is important to understand the approximations used in deriving equations that critically limit the utility of this traditional approach.

1.1 Importance of kinetic analysis

The field of kinetics is essential to understand biology and chemistry because the rates of reaction reveal the mechanism. Too often, investigators propose a reaction mechanism based on equilibrium measurements or inspection of static structures. In doing so, they overlook a fundamental tenet of thermodynamics: the net free energy change for a reaction is independent of pathway. A corollary of this law is that equilibrium measurements cannot define the sequence of reactions. Structures provide a wonderful glimpse into the workings of proteins and nucleic acids, but they represent the beginning, not the end, of investigations to determine mechanism.

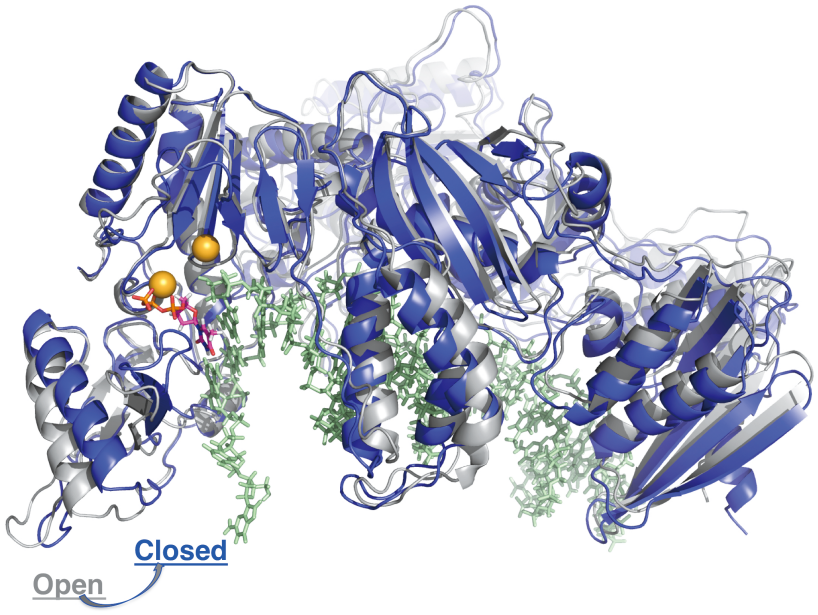

Figure 1.1 *Conformational dynamics of HIV reverse transcriptase.* This figure shows the structures of HIVRT (HIV reverse transcriptase) before (grey) and after (blue) nucleotide binding from molecular dynamics simulations.[118] These studies complemented kinetic analysis to establish that the conformational change is a primary determinant of accuracy in DNA replication catalyzed by HIV reverse transcriptase.[112] Reproduced with permission from Kirmizialtin *et al.* (2012).[118]

Consider the structure of HIV reverse transcriptase (Figure 1.1), showing the transition from open to closed states after the binding of a nucleoside triphosphate. Observations of conformational changes after substrate binding are suggestive of models where a substrate-induced change in structure aligns residues for efficient catalysis of a specific reaction. However, the role of induced-fit in enzyme specificity has been controversial since it was first proposed in 1957,[122] largely because of the vagueness of the model, the lack of definitive kinetic data, and the inability of structural data to establish a pathway.

Structural studies alone cannot address the question because specificity is a kinetic phenomenon. Recently, direct kinetic measurements of each step in the reaction pathway resolved the controversy and established a new paradigm for enzyme specificity in which the substrate-induced conformational change is the major determinant of enzyme specificity due to kinetic partitioning of the closed state.[112] Molecular Dynamics (MD) simulations complemented these studies to reveal the structural transitions responsible for the observed kinetics and specificity.[118] As described in detail in Chapter 12, this example illustrates how simple and direct kinetic measurements can provide definitive answers to long-standing questions—a recurrent theme throughout this text.

This book provides the fundamental principles that guide the design and interpretation of insightful experiments to explore the relationships between structure, function, and dynamics. What characterizes an insightful experiment? The right experiments: (1) dive straight to the heart of a scientific question rather than dance around it, (2) can be interpreted rigorously to provide new insights, and (3) provide a quantitative basis to distinguish alternative theories. You should not choose to perform an easy experiment if the results are unlikely to yield new insights. It is better to perform a difficult experiment that is easy to interpret and gives an unequivocal answer. Here we strive to teach you to recognize, design, and rigorously interpret the right experiments, and to analyze experimental observations globally to define a unique, unifying model to account for all available data.

1.2 Computer simulation

In this book, computer simulation using *KinTek Explorer* software provides the foundation for teaching kinetics and fitting data based on numerical integration of rate equations.[105] This software provides a means to help develop an intuitive understanding of kinetics as well as a method for rigorously fitting data. We encoded in the design of this software the logic behind designing experiments as a basis for modeling and rigorously interpreting the results. The process of setting up an experiment in the software mimics the protocols for experiments in the laboratory. *KinTek Explorer* software provides a *dry lab* in which students can perform experiments computationally and analyze results to test a chosen model; it provides opportunities to practice both designing and interpreting experiments without getting your hands dirty.

As a companion to this book, *KinTek Explorer* software can be downloaded at https://www.kintekexplorer.com and run in student mode without a license. If your work involves collecting and analyzing kinetic and/or equilibrium data, then you can purchase a license that enables you to input data and export results in publication-quality format. On the website, you can also find an instruction manual and numerous video tutorials to help you get started. In this book, we do not spend much time on how to use *KinTek Explorer* because that information is provided with the software.

Rather, we concentrate on the principles behind the design of experiments and data fitting based on numerical integration of the rate equations.

Most importantly, all-too-common errors of interpretation can be avoided when data are fit using computer simulation because the program forces you to specify the starting conditions, identify the signal being measured, and account for the rate *and* amplitude of the reaction without making any approximations. For example, Roger Goody[71] has clearly described errors in interpreting kinetics of GTPases and guanine nucleotide-exchange factors that are easily resolved by computer simulation using *KinTek Explorer*. Conventional data fitting is focused on estimating the rate of the reaction when fitting data using a simplified equation, while largely ignoring the starting conditions for the experiment and the amplitudes of the reaction.

Figure 1.2 illustrates the concepts behind computer simulation based on numerical integration of rate equations. We can easily enter a model and a set of rate constants, then define an experiment by specifying the starting concentrations of reactants and a mathematical description of the observable signal. *KinTek Explorer* then displays the expected results. In this example, we simulate a simple two-step reaction where enzyme (E) binds substrate (S), and then the enzyme-substrate complex (ES) isomerizes to a new state, (EX).

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EX$$

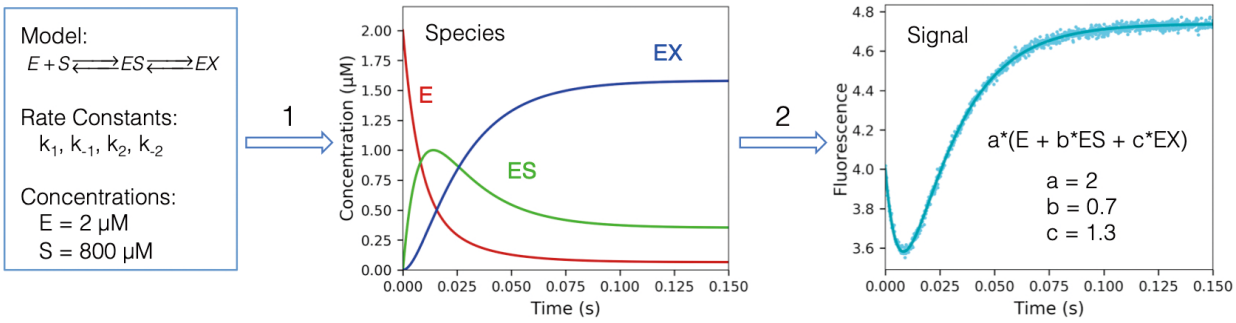

Figure 1.2 *Numerical Integration.* This diagrams illustrates the two steps in simulating a reaction. 1. Starting with a model, rate constants and concentrations, numerical integration of the rate equations yields the time-dependence of each species. 2. The time dependence of species is translated to an observable signal by a simple mathematical description.

To generate an output signal, we start with a model and a set of rate constants and then define an experiment by specifying the initial concentrations of reactants. The program then uses numerical integration to calculate the time dependence of each species. Next, the output signal is calculated as a mathematical description, in this case, as a weighted sum of species to mimic a fluorescence change. In fitting data, we take an observable signal and find a model and parameters that reproduce the data. This exercise reveals the relationships between observable signals, the underlying models, and the details of a given experiment. In fitting data, observable signals are interpreted to suggest a plausible model, which we then enter into the software along with details of the experiment to test whether the model and a set of parameters can reproduce the original data.

Alternative models can be considered to seek a *minimal model*, defined as one that is necessary and sufficient to account for the observations. In

order to eliminate overly complex models, we perform confidence contour analysis, a process by which each parameter is varied systematically to quantify the extent to which it is defined by the data.[104] The simulation software can also be used to help guide the design of new experiments to distinguish alternative models.

1.2.1 Dynamic simulation

One unique feature of *KinTek Explorer* software is the use of *dynamic simulation*, where the user can modify a rate constant, starting concentration, or output scaling factor by scrolling up and down with the computer mouse while simultaneously observing the changes in predicted time- and concentration-dependence of an observable output. This *dynamic simulation* is useful for learning kinetics but also facilitates exploration of parameter space to find starting estimates of kinetic parameters for fitting by nonlinear regression.

To illustrate the use of dynamic simulation, we consider a simple model for binding substrate (S) to an enzyme (E):

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

As described in more detail in Section 1.5 (page 26), rate constants k_1 and k_{-1} for the forward and reverse reactions, respectively, define the rate of change in concentrations of the reactants and products. The rate of formation of ES is given by the difference between the forward and reverse rate, each of which is the product of the rate constants and concentrations.

$$d[ES]/dt = k_1[E][S] - k_{-1}[ES]$$

We can expect the observed rate of formation of ES to increase in proportion to the substrate concentration with a coefficient defined by k_1. We will wait until Chapter 8 to derive equations for the time dependence of the reaction, but it is easy to explore the relationships between rate constants and the observable signal using computer simulation.

Figure 1.3 shows the simulation of this simple one-step binding reaction at various concentrations of substrate monitored by a change in protein fluorescence. Using *KinTek Explorer* software, you can open the example file, *Binding-onestep-example.mec*, which can be found in the *Examples* folder with the software. The model is based on a 30% increase in protein fluorescence to provide a signal for substrate binding.

When running *KinTek Explorer*, if you click on a rate constant or concentration with the computer mouse, you can alter its value by dragging the mouse up and down. As you do so, the computer rapidly recomputes and displays the time course of the reaction so you can see the relationships between these parameters and an observable signal. Note that the exponential decay rate increases as a function of increasing concentration of substrate, as evidenced by the shortening half-life. The forward and reverse rate constants can also be scrolled to see the change in shape of the curves. Increasing the rate constant for the reverse reaction (k_{-1}) decreases

the amplitude of the reaction by decreasing the equilibrium constant. Increasing the forward rate constant (k_1) leads to an increase in the decay rate and amplitude of the reaction. By simultaneously fitting the rates and amplitudes of the reactions, both rate constants and the fluorescence scaling factors can be defined.

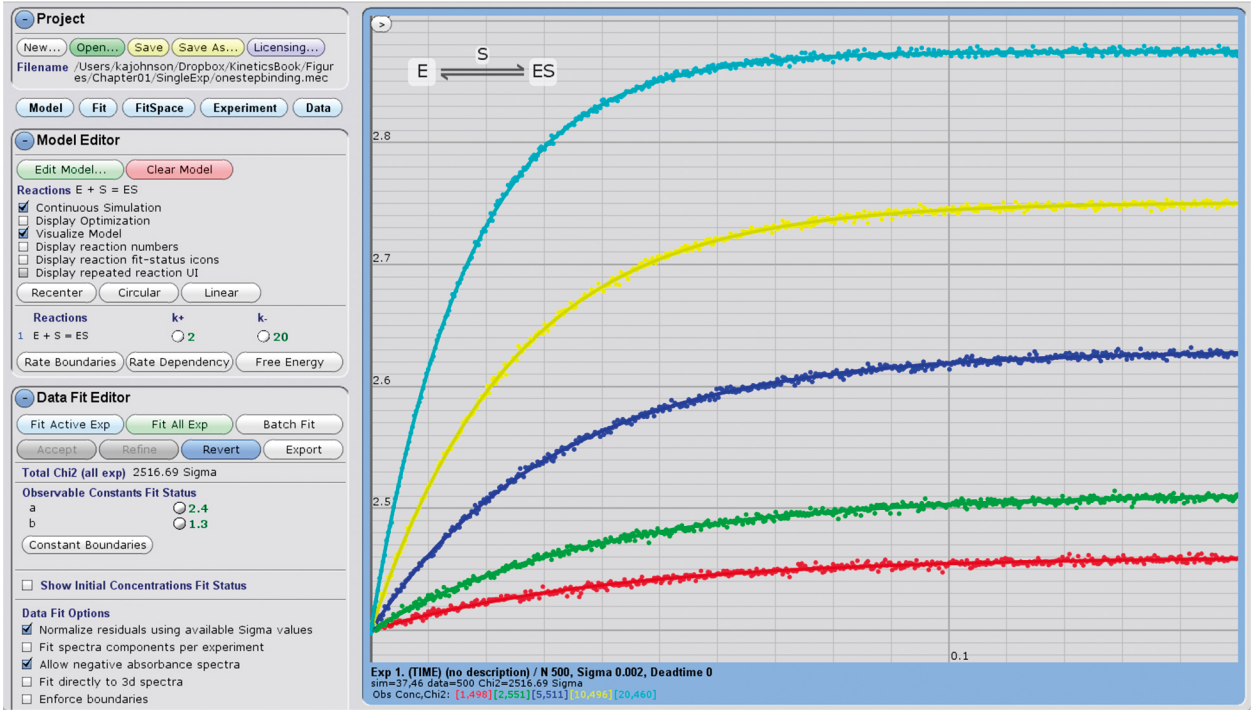

Figure 1.3 *Kinetics of substrate binding.* Substrate binding to an enzyme was simulated for a simple one step reaction by mixing 1 μM enzyme with 1, 2, 5, 10 and 20 μM substrate. We modeled a 30% increase in protein fluorescence on substrate binding using the output function $a * (E + b * ES)$, with $a = 2.4$ and $b = 1.3$.

The purpose of this exercise is to help you to develop an intuitive understanding of how a given model and rate constants are revealed by the data, and conversely, how data can be used to define a likely model. Greater rate constants for the forward reaction, k_1, lead to faster rates of increase in fluorescence. Increasing the rate constant for the reverse reaction, k_{-1}, decreases the amplitude and therefore decreases the half-time of the approach to equilibrium. In a sense, the reaction does not have as far to go, so it reaches the endpoint in less time. This phenomenon is discussed in detail in Chapter 8. For now, this example serves only as an illustration of things to come.

Dynamic simulation is used throughout the book to illustrate the relationships between rate constants, starting concentrations, output scaling factors, and observable signals. In subsequent chapters we will explore these relationships mathematically. However, the use of dynamic simulation gives you a kind of biofeedback where a given action—a change in rate constant, starting concentration or scaling factor—yields an immediate response visible through changes in the output signal. Through repetition, this exercise helps to cultivate an intuitive understanding of how mechanisms are revealed through patterns in the kinetic data. Ultimately, the goal of kinetic analysis is to deduce a model based on analysis of data, but we begin by going in the opposite direction. Once we understand how a

given model generates predictable patterns in data, we can then propose a plausible model based on inspection of the data.

1.3 Information content of kinetic data

Here we introduce the concept of the *information content* of kinetic data, which we define as the maximum amount of mechanistic information that can be extracted from a given data set. Understanding the information content that can be obtained from a given experiment is important since it sets the boundaries of expectations for what can be learned from one experiment and what additional experiments may be needed to address unanswered questions. Trying to extract more information than can be supported by the data results in frustration and possible embarrassment. On the other hand, failing to recognize the full extent of information available from a data set leads to wasted time and resources.

Major goals of this book are to teach you to understand the information content of a given experiment and to teach you how multiple experiments can come together to answer questions that cannot be addressed by any single experiment. Developing an intuitive understanding of the information content of an experiment can be achieved through two intertwined approaches. First, for simple systems the derivation of equations can readily reveal the information content of an experiment in terms of the maximum number of parameters that can be defined in fitting the data using the appropriate equation. For example, because steady-state kinetic data can be reduced to an equation defined by a straight line on a double-reciprocal plot (see Section 5.4.3, page 159), it is indisputable that the data can be represented by only two parameters. The intercept and slope of the plot give $1/k_{cat}$ and $1/(k_{cat}/K_m)$, respectively.

Equations cannot be solved for more complex systems, and even relatively simple systems require significant approximations to derive the equations. In fitting data, each experiment requires a different set of equations (see ahead, Figure 1.10, page 37), which makes it difficult, if not impossible, to fit multiple experiments simultaneously. Accordingly, we use computer simulation to model and fit the data. Dynamic simulation provides initial assessment of the extent to which individual rate constants may be constrained, and therefore defined, by the data. After achieving a good fit to the data, confidence contour analysis defines the *information content* of a given data set to quantify whether the model and all of the parameters are supported by the data.

The information content depends on the underlying mechanism, intrinsic rate constants, and details of the experimental design. One of the difficulties of kinetic analysis is that a given experiment may have a different information content for the same model depending on the underlying rate constants. Computer simulation assists in resolving these complexities.

1.4 The role of models in biochemistry

Ever since Copernicus developed mathematical models that could be used to predict the motions of the stars and planets,[44] science has progressed through careful observation interpreted with the use of mathematical models. These models for the basis for predictions that can be tested using more accurate measurements and new techniques. The year 2013 marked the 100th anniversary of publication of the classic Michaelis-Menten paper,[153] which provided the first evidence for the existence of an enzyme-substrate complex formed during catalysis. Recently, the author and Roger Goody published a complete English translation of the Michaelis-Menten paper (published in German), and offered insight into the important scientific questions and analysis methods of the day.[103]

Michaelis and Menten defined kinetic parameters for a minimal enzyme pathway for the hydrolysis of sucrose to form fructose and glucose catalyzed by the enzyme invertase (Figure 1.4), so named for the inversion of the optical rotation that provided a signal to measure the rate of reaction.

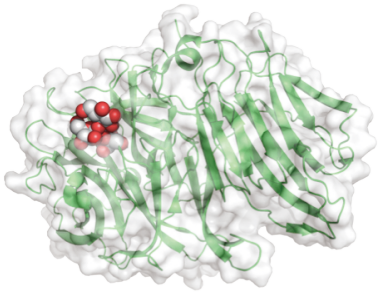

Figure 1.4 *Structure of invertase.* The structure of invertase was published 100 years after the landmark Michaelis-Menten paper. Drawn using Pymol by alignment of PDB ID:4EQV[181] with the substrate from PDB ID:2AEZ.[214]

Through kinetic analysis, Michaelis and Menten successfully addressed the pressing enzymology question of their time: can the observed rates of catalysis be explained by a model in which the rate is proportional to the concentration of a theoretical enzyme-substrate complex? Their simple model made the prediction that the substrate (S) binds in a rapid equilibrium with the enzyme, which then catalyzes the reaction and releases the products, fructose (F) and glucose (G).

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + G + F$$
$$v = \frac{V_{max}[E][S]}{K_m + [S]} \qquad (1.1)$$
$$V_{max} = k_{cat}[E]$$

1.4.1 Lessons from Michaelis and Menten

The Michaelis-Menten model illustrates the general importance and properties of mathematical models that apply to research today.[95] The important question for Michaelis and Menten was whether the rate of the enzyme-catalyzed reaction was proportional to the concentration of enzyme-substrate complex predicted by a simple substrate-binding model. Michaelis and Menten raised the standard of proof even higher by insisting that the model account for the full progress of the reaction to completion, not just the initial velocity.[153,103]

The Michaelis-Menten paper illustrates the important roles that models play in understanding biology and chemistry:

1. *The analysis provided evidence for the existence of something that could not be seen!* The nature of enzymes was not known at the time, and consequently, the concentration of an enzyme could not be determined. Nonetheless, the data and mathematical analysis provided evidence that the enzyme-substrate complex must exist, because the rate measurements over a range of starting concentrations and reaction times could

be explained based on a simple model used to calculate the concentration of the enzyme-substrate complex. Moreover, K_m, the Michaelis constant, so named by subsequent authors, provided an estimate of enzyme-substrate binding affinity. We now know that the Michaelis constant does not equate to substrate binding affinity, but their discovery seeded the century of studies that followed to more accurately establish the kinetic and thermodynamic basis for catalysis.

2. *Importance of quantitative analysis!* Michaelis and Menten derived equations for product inhibition and used initial velocity methods to measure K_i values for the fructose and glucose. In the final test of their model, they included product inhibition in their analysis of the full reaction time course. This provided the most stringent test of the postulate that the rate of reaction was proportional to the concentration of a hypothetical ES complex.[153,103] A hundred years later, analysis of Michaelis and Menten's data using computer simulation methods reveals that it would not be possible to account for the full progress curves without including the effects of product inhibition. Although this is not explicitly stated in their paper, it is likely that their first attempts failed to achieve a satisfactory fit, and they then realized that the deviation of the curves reflect product inhibition. By including product inhibition in their comprehensive model, the global constant (V_{max}/K_m) derived by Michaelis and Menten is identical to the value derived today by globally fitting their data using computer simulation. This is a testament to their care and diligence in fitting data using only a pencil and paper! It also illustrates the importance of accurate, quantitative analysis to test a proposed model.

3. *Testing the model required attention to experimental details!* In earlier work, Henri[82] had attempted to test the same model, but he neglected to control for variations in pH and failed to account for the slow mutarotation of glucose (equilibration of the α and β anomers). Thus, his data were too inaccurate to support the proposed model. Michaelis and Menten controlled pH with an acetate buffer and stopped the reaction with alkali to increase the rate of mutarotation before making measurements. By understanding the chemistry, Michaelis and Menten minimized uncertainties in the experimental setup to afford a more accurate test of the model.

4. *The model is an approximation of reality!* Effective models contain the minimal number of features to account for available data. For example, Michaelis and Menten knew that pH was important, but pH does not appear anywhere in their model. More importantly, the chemical conversion of substrates to form products at the active site of the enzyme is a necessary step that is omitted from the model, largely because the data could not define the rate of reaction at the active site so the simpler model was sufficient. Finally, the reaction was written with irreversible product release, even though it was known that the products of the reaction could rebind to the enzyme. Thus, the model was simplified to a

level that could be supported by the initial velocity data, and therefore, its development clarified the then-current state of knowledge.

5. *The model informs us how to better perform the experiments!* The model stated that the rate of turnover would change as a function of time as the substrate was depleted and products accumulated to inhibit the enzyme. But it also implied that in the initial phases of the reaction, there would be a brief period during which the substrate concentration was largely invariant. Thus, initial velocity measurements based on a separation of time scales allowed focus on a phase of the reaction during which a simplified measurement could provide meaningful results. The simplified data analysis relied on these critical approximations: product release is irreversible, and $[S]$ does not change significantly during the initial phase of the rate measurement. This keen observation formed the foundation of enzyme kinetic methods for much of the century that followed their publication.

6. *The model drives the development of new methods to test predictions!* The most important aspect of a model is that it makes predictions that can be tested through new quantitative measurements. Thirty years after the Michaelis-Menten paper, Britton Chance performed the first stopped-flow experiments to directly observe the formation and decay of an enzyme-bound intermediate using an optical signal observed with horseradish peroxidase. Thus direct measurement of an enzyme-bound species confirmed the Michaelis-Menten model.[33]

It is important to recognize that models represent approximations to reality and to acknowledge the role that those approximations play. A model should only be as complex as the data can support. Thus, in fitting data, an important requirement is that it is based on a minimal model sufficient to account for the data. As additional data are included, the model can then be expanded to include the new information.

As we increase the sophistication of measurements and the power of computer-based modeling, we can generate models of increasing complexity and fit multiple experimental data sets to a single, unifying model. Now the challenge is to ensure that the model represents the minimal number of steps and intermediates required to account for all available data and preclude over-interpretation. In the pages that follow, we provide an intuitive visual guide to assess the quality of data fitting, define the extent to which parameters are constrained by the data, and ultimately evaluate whether the data support or refute a given model.

1.5 Conventions regarding rate constants

First, we introduce the fundamental concepts of kinetics and nomenclature illustrated by the simple enzyme pathway shown below, with one substrate, one intermediate (EX), and one product. This approach can be easily expanded to include enzymes with multiple substrates and products or

applied to chemical kinetics without enzymes.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EX \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EP \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + P \qquad (1.2)$$

Rate constants are designated by a *lowercase* k_n, where the n is the n^{th} step in the pathway, with a positive integer for the forward reaction and a negative integer for the reverse. Equilibrium constants (K_a or K_d) and Michaelis constants (K_m) are defined by a ratio of rate constants and are designated by an *uppercase* K. In this example, there are six first-order rate constants (black) and two second-order rate constants (red).

Note that in fitting data, we seek a minimal model sufficient to account for available data so each rate constant defines a kinetically significant step in the reaction. A given kinetic step could be a function of several microscopic steps. For example, if $k_3 \gg k_2$ and $k_{-2} \gg k_{-3}$, then the intermediate, EX, breaks down faster than it is formed in each direction, and it will not be observed. In this case, which is common in enzymology, the scheme collapses to a minimal pathway that omits EX even when you know it must exist chemically.

First-order rate constants involve the reaction of a single species and have units of s^{-1}. For example for the simple model:

$$\text{For: } ES \xrightarrow{k_{-1}} E + S \text{ the velocity is } \frac{d[ES]}{dt} = -k_{-1}[ES]$$

Second-order rate constants involve the collision of two species and have units of $M^{-1}s^{-1}$.

$$\text{For: } E + S \xrightarrow{k_1} ES \text{ the velocity is } \frac{d[ES]}{dt} = k_1[E][S]$$

It is convenient to use $\mu M^{-1}s^{-1}$ or sometimes $nM^{-1}s^{-1}$, depending on the concentration scale of your data. Second-order rate constants are limited by diffusion to less be than $\sim 10^9 M^{-1}s^{-1} = 1000\ \mu M^{-1}s^{-1} = 1\ nM^{-1}s^{-1}$.

Rate constants are essentially coefficients that define the linear relationship between the rate of reaction and concentrations of reactants. For example:

$$k_1 = \frac{d[ES]/dt}{[E][S]} \qquad \text{Units of: } \frac{\mu M/s}{(\mu M)(\mu M)} = \mu M^{-1}s^{-1}$$

Pseudo-first-order rate constants include the concentration of one species in a bimolecular reaction and have units of s^{-1}; this simplification is valid only when one species is in excess over the other. For example, when the starting concentration of $[S]_0 \gg [E]_0$, we make the approximation that the concentration of S is constant so E decays with a pseudo-first-order rate constant given by $k_1[S]$.

$$E \xrightarrow{k_1[S]} ES \text{ so that } d[ES]/dt = k_1[S][E]$$

This is a critical approximation that is needed to solve differential equations describing the reactions as described in Eqn 8.2 on page 224. In Chapter 2 we show that this limitation is overcome by the use of computer simulation based on numerical integration of the rate equations. However, in fitting data using equations, the concentration of substrate must be at least fivefold greater than the concentration of enzyme. Experiments can also be performed with enzyme in excess over substrate so the substrate decays at a rate governed by $k_1[E]$ as described in Section 11.1 (page 318).

Finally, the net rate of change in the concentration of ES is the difference between the forward and reverse rates.

$$\text{The model: } E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{ES} \text{ gives } d[ES]/dt = k_1[E][S] - k_{-1}[ES]$$

1.5.1 Initial velocity versus eigenvalue

As we describe in more detail in Chapter 8, the time dependence of disappearance of free enzyme follows a single exponential as illustrated in Figure 1.5, provided that the concentration of substrate is in sufficient excess over the enzyme.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{ES}$$
$$[E] = ([E]_0 - [E]_\infty) \cdot e^{-\lambda t} + [E]_\infty \qquad (1.3)$$
$$\lambda = k_1[S] + k_{-1}$$

where $[E]_0$ and $[E]_\infty$ are the concentrations of enzyme at time zero and in the approach to infinity, respectively, and λ is the parameter that governs the time dependence of the exponential decay and is termed the "eigenvalue." Note, λ is not a rate constant; rather, it is a function of two rate constants and a concentration, in this example. We will also refer to the eigenvalue as the exponential "decay rate" because it describes the decay of E over time, analogous to radioactive decay.

Kinetic measurements are based on monitoring the change in concentration of a reactant, intermediate, or product as a function of time. Signals are provided by direct measurement of the concentration, or by absorbance or fluorescence. In any case, one wants to obtain a measure of the rate of change of individual species as part of studies to establish the underlying mechanism.

There are two methods for measuring the speed of a reaction, either as an initial velocity—a slope giving units of concentration/time—or as a fit to an exponential function (Eqn 1.3) to give the eigenvalue (λ) which has units of 1/time, as shown in Figure 1.5.

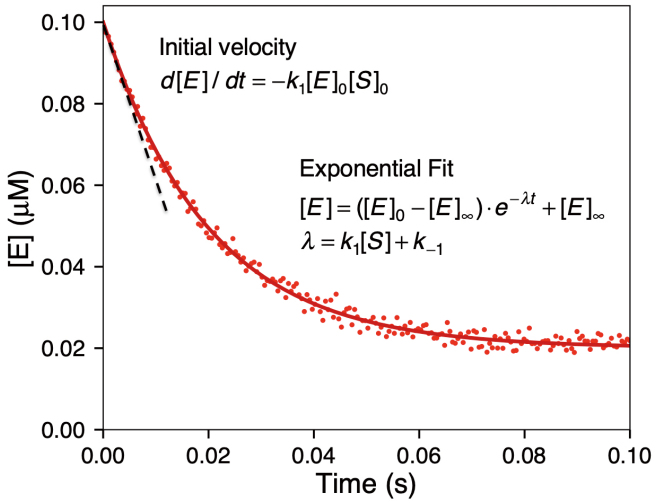

Figure 1.5 *Measuring the rate of a reaction.* This figure shows the exponential decay of free enzyme upon binding substrate. The data were simulated with 0.1 μM enzyme, 20 μM substrate and with a second-order rate constant, $k_1 = 2\ \mu M^{-1}s^{-1}$ and a reverse rate constant of $k_{-1} = 10\ s^{-1}$.

$$E + S \underset{10\ s^{-1}}{\overset{2\ \mu M^{-1}s^{-1}}{\rightleftharpoons}} \text{ES}$$

Fitting the data in Figure 1.5 to a single exponential function (Eqn 1.3) yields an observed decay rate (eigenvalue) of 50 s^{-1}, which is defined by the product of the forward rate constant times the substrate concentration plus the rate constant for the reverse reaction. This process of data fitting yields the lifetime ($1/\lambda$) of free enzyme at a particular substrate concentration and is independent of the starting enzyme concentration. $\lambda = k_1[S] + k_{-1} =$

$50\ s^{-1}$ In contrast, fitting to derive the initial rate (extrapolated to time zero) yields a slope with units of concentration/time. The measured value depends on the rate constant, k_1, and the initial concentrations of substrate and enzyme: initial velocity $= d[E]/dt = -k_1[E]_0[S]_0 = 4\ \mu M s^{-1}$

You can use either initial velocity or exponential fits to measure how fast the reaction proceeds, but there are major differences:

- Fitting to an exponential function includes more data, provides a more accurate assessment of the model, and affords more precision in estimating the rate constants. Fitting data to derive initial velocities is error-prone because of the restricted time interval and smaller amplitude of change in signal.
- In this example, the eigenvalue is a function of the sum of the forward and reverse rate constants and the concentration of the species in excess, S in this case, $\lambda = k_1[S] + k_{-1}$. In general, the eigenvalue is a complex function of all of the rate constants contributing to the final endpoint, but does not depend on [E] when [S] is in excess.
- The initial velocity is a function of the product of the forward rate constant and concentrations of the two reactants *at the time of the measurement*; for example, $d[E]/dt = -k_1[E]_0[S]_0$.
- Initial velocity measurements include y-axis scaling factors and $[E]$. For example, the initial slope of a fluorescence signal will have units of fluorescence/time. In contrast, in fitting to an exponential function, the y-axis scaling factors are isolated in the amplitude terms and do not affect the decay rate estimate.

1.5.2 Rate constant or eigenvalue?

Confusion arises in the nomenclature often applied to the results of fitting data to an exponential function. In the early days of enzymology, the term *rate* was defined to describe a change in concentration over time as the initial velocity, $d[P]/dt$. When data were fit to an exponential function, the exponential term was called a *rate constant.* While the "rate" changes as reactants are depleted, the "rate constant" does not. *However, use of "rate constant" to described an eigenvalue is a major mistake because "rate constant" is more stringently defined to represent a single step in a pathway.* A fit to an exponential function yields a value that is most often a complex function of intrinsic rate constants. Therefore, it is inaccurate and misleading to call an eigenvalue a rate constant.

In order to avoid confusion, the term *rate constant* must be reserved to refer only to a single reaction step in a pathway as described by a *lowercase* k over an arrow: $A \xrightarrow{k} B$.

Unfortunately, some investigators still espouse the use of the term *rate constant* for the value derived from an exponential fit (units of 1/time) to distinguish it from a rate measured as product formed versus time (units of signal change over time) only because of historical use.[14] Because of the widespread misuse of the term *rate constant* to represent an observed decay

rate derived by fitting to an exponential function, we give two examples of the confusion it generates.

We have already discussed the simple model involving the reversible binding of substrate to an enzyme as illustrated in Figure 1.5.

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

$$\begin{gathered}[E] = ([E]_0 - [E]_\infty) \cdot e^{-\lambda t} + [E]_\infty \\ \lambda = k_1[S] + k_{-1}\end{gathered} \tag{1.4}$$

The observed eigenvalue, $\lambda = k_1[S] + k_{-1}$, is certainly NOT a rate constant. In fact, it is not even a constant because it is a function of substrate concentration. This illustration is for the simplest pathway. As pathways expand, the eigenvalue gets far more complicated, and the confusion in misusing the term *rate constant* to describe an eigenvalue gets worse.

Consider a pre-steady-state burst experiment using the following simple model, which is described in Chapter 10.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \xrightarrow{k_3} E + P$$

The time dependence of product formation follows a single exponential followed by a linear phase, leading to steady-state turnover at a rate k_{ss}.

$$\frac{[P]_{obs}}{[E]_0} = \frac{[EP] + [P]}{[E]_0} = A_0(1 - e^{-\lambda t}) + k_{ss}t$$

If we make the simplifying approximation that substrate binding is a rapid equilibrium, then the observed decay rate (eigenvalue) is defined by:

$$\lambda = \frac{K_1[S]}{1 + K_1[S]}k_2 + k_{-2} + k_3$$

Clearly, λ is *not* a rate constant! The very real confusion comes with the statement that "fitting the pre-steady-state burst to an exponential function defines the *observed rate constant* for the chemical reaction at the active site." It does not! It defines the *eigenvalue*: the exponent is a function of all of the rate constants in the pathway, including product release and the reverse of the chemistry step!

Another example is a simple two-step binding reaction where the data follow a double exponential function with two eigenvalues (see Section 9.3, page 278).

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EX$$

$$Y = A_\infty + A_1 \cdot e^{-\lambda_1 \cdot t} + A_2 \cdot e^{-\lambda_2 \cdot t}$$

The two eigenvalues are derived from the roots of a quadratic equation:

$$\lambda_{1,2} = \frac{(k_1[S] + k_{-1} + k_2 + k_{-2}) \pm \sqrt{(k_1[S] + k_{-1} + k_2 + k_{-2})^2 - 4 \cdot (k_1[S] \cdot (k_2 + k_{-2}) + k_{-1}k_{-2})}}{2}$$

The complexity of the problem is obviously underestimated by a description of the two eigenvalues as "observed rate constants"—and this is for a reaction involving only two steps!

1.5.3 Do not use "observed rate constant"

Using the term *observed rate constant* to describe an eigenvalue is akin to describing the Michaelis constant as a measure of the *apparent substrate binding affinity*. In the minds of students and too many research scientists, that statement gets truncated into the belief that the Michaelis constant *is* a measure of substrate binding affinity—never mind the important qualifying adjective, "apparent." Using the term "observed rate constant" is even worse since the qualifying adjective "observed" is even weaker than "apparent" in raising a flag of caution for interpretation of the measured parameter. After all, are not all measured values "observed" parameters?

Learning kinetics is hard enough without the confusion of dual meanings of the term *rate constant*, which seems to be applied to eigenvalues only because of historical precedence. *Now is the time to change this mistake*. Continued use of the term *observed rate constant* to refer to an eigenvalue blurs important distinctions that must be maintained. Eigenvalues are dependent on the conditions of the experimental setup, including concentrations of reactants and products and the methods of measurement. This alone should be sufficient reason to discard this outdated nomenclature.

The distinction between an *eigenvalue* and a *rate constant* is increasingly important as we consider different methods of fitting data. Conventional fitting using equations yields net decay rates or eigenvalues, which must then be interpreted to define the underlying rate constants, often requiring inclusion of amplitude information and/or substrate concentration dependence of observed rates. More importantly, modern methods of data fitting based on computer simulation directly yield individual rate constants defined by a given model. So it is all the more important to understand that fitting data based on an equation does not yield a rate constant, except in rare circumstances of irreversible reactions, as described on page 264.

1.5.4 Use of the word "rate"

In this book we insist on a standard where the term "rate constant" is reserved used to refer only to the rate coefficient for a single step in a reaction pathway. So we are left with a problem of semantics. How should we refer to the speed of a reaction when it is measured by fitting data to an exponential function as in Figure 1.5? If we reserve the word "rate" to only refer to a measurement of velocity as in $d[P]/dt$, and we cannot use the term "observed rate constant", then we are left with awkward sentence structure in referring to the parameter derived in fitting data to an exponential function. For example, Cornish-Bowden proposed adopting the phrase "frequency constant,"[45] which has not been widely used. Perhaps "decay constant" would be a better alternative.

Word usage evolves in response to environmental changes as certainly as do Darwin's finches on the Galapagos Islands.[218] Enzymology has advanced from a reliance on initial velocity measurements to a growing recognition of the importance of transient-state kinetic methods where the speed of the reaction is measured by fitting to an exponential function. We are now in

an era where data fitting based on simulation affords rate constants directly, not eigenvalues or initial velocities. The word "rate" has been increasingly used generically to refer to the speed of a reaction *independent of how it was measured*, not because of ignorance,[14] but out of a desire to concisely describe the speed of a reaction. When having to choose between the dichotomy over the use of the word "rate" versus confusion over the use of the term "observed rate constant," the latter is rejected because it is the most misleading descriptor of information content.

1.5.5 The new standard

In this book, we focus on methods that yield true rate constants directly in the process of data fitting by simulation. This is a big step forward from traditional equation-based data fitting, which yields eigenvalues that are a function of multiple rate constants and concentrations. For that reason, it is important to make the clear distinction that an observed decay rate is not a rate constant. We adopt the following conventions:

- *Rate constant* is a term strictly reserved to refer to a single reaction step as defined by a lowercase k over an arrow in a pathway, $E+S \xrightarrow{k_1} ES$. It must not be used in any other context. In reading the literature, beware of the use of the term "observed rate constant" and carefully interpret the intended meaning in context. Are the authors referring to a rate constant or an eigenvalue?
- *Rate or velocity* refers to a change in concentration with time such as in an initial velocity measurement of product formation; for example, $v = d[P]/dt = k_4[EP]$ for Scheme 1.2.
- *Rate* is also used generically to refer to the speed of a reaction independent of how it was measured whenever it is not necessary to make the distinction of how the reaction was measured. Take care not to use "rate" when you mean "rate constant," the latter being more specific and informative. More importantly, do not use the phrase "observed rate constant" when you mean "eigenvalue."
- We specifically use the term *eigenvalue* or *decay rate* when it is necessary to emphasize that a reaction rate estimate was derived by fitting to an exponential function, such as: $[E]/[E]_0 = e^{-\lambda t}$. The symbol λ is used in equations to designate the eigenvalue.
- In addition to *eigenvalue* or *decay rate*, two other terms are mathematically defined based on the eigenvalue:

  The *lifetime* is defined by τ *(tau)* $= 1/\lambda$.

  The *half-time* is defined by $t_{1/2} = ln(2)/\lambda$.

1.6 Three timescales of enzyme catalysis

Experiments performed using different enzyme concentrations and different timescales yield very different information. To illustrate this, we use

the computer simulation software to generate synthetic data that mimics experiments performed under different conditions based on the model shown in Scheme 1.5. At this point, it is not expected that a beginning student fully understands all of the details given here. This section provides a preview of where we are headed and is intended only to illustrate the numerous approaches that we expand on in subsequent chapters.

The following model and rate constants were used to generate data under various conditions and timescales to illustrate the types of experiments that can be performed, as shown in Figure 1.6.

$$E + S \underset{500\,s^{-1}}{\overset{10\,\mu M^{-1}s^{-1}}{\rightleftharpoons}} ES \underset{20\,s^{-1}}{\overset{150\,s^{-1}}{\rightleftharpoons}} EP \underset{0.02\,\mu M^{-1}s^{-1}}{\overset{5\,s^{-1}}{\rightleftharpoons}} E + P \qquad (1.5)$$

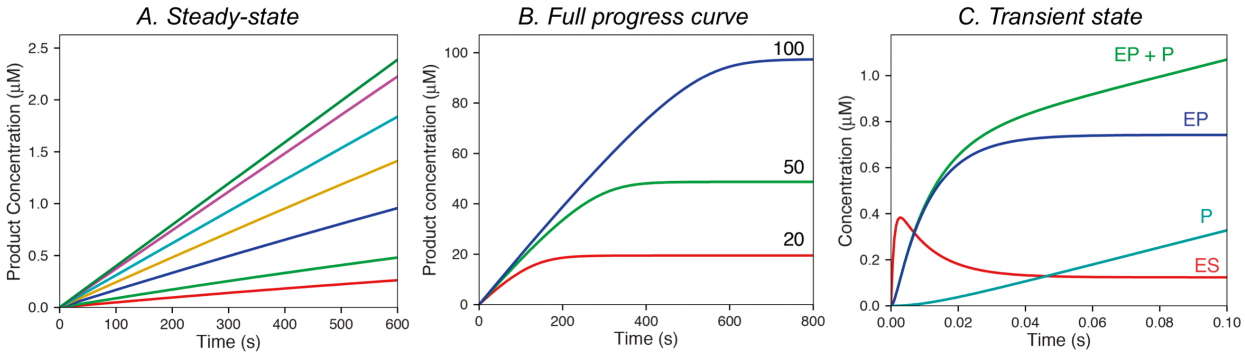

Figure 1.6 *Three timescales of enzyme catalysis.* A. Steady-state turnover measured at low enzyme concentration over a 10 min timescale. B. Full progress curves at a slightly higher enzyme concentration and longer time. C. Transient-state kinetics using a high enzyme concentration and 10 ms timescale.

Figure 1.6A: Steady-state kinetics are measured at a low concentration of enzyme (0.001 μM) and several concentrations of substrate (1-100 μM). Measurement of the concentration dependence of the initial rate affords estimates of k_{cat} and K_m.

Figure 1.6B: Following the reaction to completion (with 0.05 μM enzyme and 20, 50, 100 μM substrate) also provides estimates of k_{cat} and K_m, but the data need to be fit by simulation and can also define product inhibition.

Figure 1.6C: Using higher enzyme concentrations (1 μM enzyme with 50 μM substrate) and shorter times provides data to define the reactions occurring at the enzyme active site in the approach to the steady state.

The only differences in the three experiments are in the starting concentrations of reactants, the nature of the observable signal, and the timescale for observation. Of course, they also yield very different information.

1.6.1 Steady-state kinetics

For decades, the study of enzyme kinetics has been dominated by steady-state methods using relatively low enzyme concentrations. Under these conditions, the concentrations of the ES and EP complexes are approximately constant over a time interval sufficient to measure the initial velocity. Measurements as a function of substrate concentration allow determination of the net enzyme turnover rates (k_{cat}) and Michaelis constant (K_m) as first described by Michaelis and Menten in 1913.[153] Note in Figure 1.7

that the initial slope of the time course increases and approaches a maximum rate as the concentration of substrate increases. The maximum rate defines k_{cat}, while the concentration dependence defines K_m. In Chapter 5 we explain the meaning of steady-state kinetic parameters. The *KinTek Explorer* mechanism file, *Steady-state.mec*, used to create these figures is available in the *Examples* subfolder with the software.

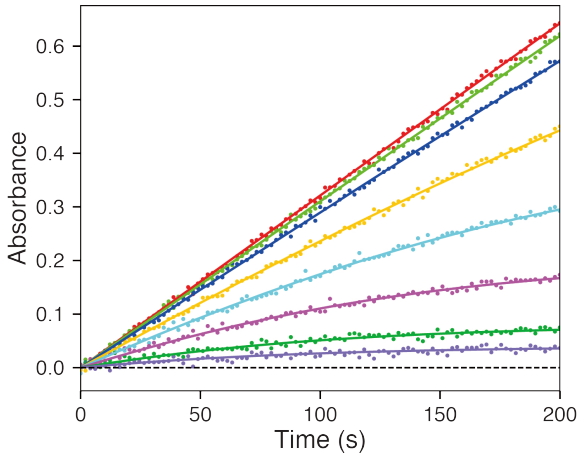

Figure 1.7 *Steady-state turnover.* Enzyme turnover was simulated using 0.01 μM enzyme and various concentrations of substrate (1, 2, 5, 10, 20, 50, 100 and 200 μM). These data can be viewed and fit in the *KinTek Explorer* example file: *Steady-state.mec*, found in the *Examples* subfolder with the software.

Note the curvature in the traces at lower substrate concentration (Figure 1.7), so we have to restrict data fitting to shorter time intervals to accurately measure the initial velocity. This constitutes a major limitation of the steady-state method due to inaccuracies at the lowest substrate concentrations. In Chapters 2 and 5 we show how the raw data from a steady-state experiment can be fit directly using computer simulation, bypassing the conventional initial velocity estimates and secondary plots of rate versus concentration to fit the rate data to a hyperbola to determine k_{cat} and K_m. Fitting by simulation bypasses the steady-state approximation and directly provides realistic standard error estimates for the steady-state kinetic parameters, as described in Section 2.5 (page 56).

1.6.2 Full progress curve kinetics

In full time course or progress curve kinetics (Figure 1.8), the reaction is followed to completion, approaching equilibrium as the substrate is converted to product. In the absence of product inhibition, one trace is sufficient to define k_{cat} and k_{cat}/K_m. This is because the decrease in velocity as substrate is depleted allows determination of the substrate concentration dependence of the rate of turnover. Thus, one time course contains as much information as the concentration series shown in Figure 1.7. Moreover, if the reaction is inhibited by the rebinding of product, the shape of the curve is altered toward the end of the reaction, and this provides additional information to define the binding affinity for the product. In this case, the experiment must be performed at several starting concentrations of substrate to sort out the effects of decreasing substrate from the effects of increasing product concentration on the rate of reaction as it approaches the endpoint. In abandoning the traditional initial velocity methods and using computer simulation for data fitting, full progress curve

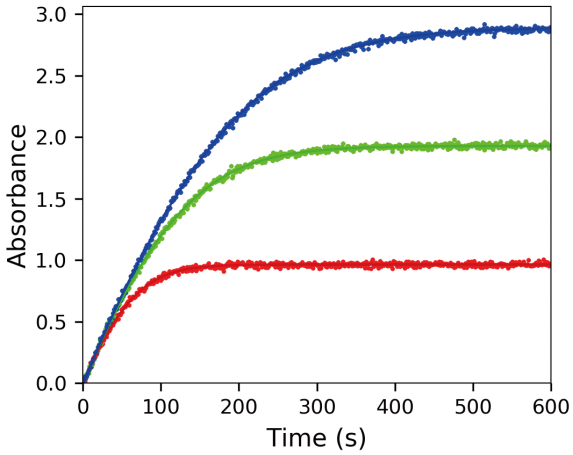

Figure 1.8 *Full progress curve kinetics.* Enzyme turnover was followed to completion using 0.1 μM enzyme with 1, 2, and 3 mM substrate, as described in *ProgressCurvesB.mec*

kinetics analysis provides much more information from fewer experiments with less wasted resources. The analysis is simpler, more direct, and produces more accurate results than traditional initial velocity methods, as described in Section 5.7 (page 170).

1.6.3 Pre-steady-state transient kinetics

The information content of steady-state and full progress curve kinetics is limited because the studies involve multiple enzyme cycles. In order to obtain information pertaining to the reactions occurring at the active site of the enzyme, it is necessary to observe reactions on the timescale of a single enzyme turnover as described in Chapters 7 to 12. Transient kinetics (Figure 1.6C) allow observation of the rates of substrate binding and the chemical reaction at the active site as well as the direct observation of enzyme intermediates. Note the rise and fall of the ES complex, coincident with the formation of the enzyme-bound product (EP). Various signals are employed to measure the reaction on this timescale. In a rapid-quench-flow experiment, the sum of product bound to the enzyme and free in solution is measured ($EP + P$), as illustrated in Figures 1.10F. For example, you can also monitor the time course of substrate binding using fluorescence methods (Figure 1.10D). Unlike steady-state kinetic analysis, where one tests various models to see which one fits the data, transient kinetic studies afford construction of a reaction pathway by putting together measurements of individual segments. For these reasons, much of this book is devoted to the use of transient-state kinetic methods. Moreover, based on the expanded knowledge derived from observing reactions occurring at the active site, we carefully consider the meaning of steady-state kinetic parameters in Chapter 5.

1.7 Data fitting—equations versus simulation

In fitting three experiments shown in Figure 1.6 using traditional methods, a different set of equations would be needed for each experiment, and each equation is based on approximations needed to solve the math, although there are no general equations for fitting full progress curve ki-

netics without significant approximations. In contrast, to fit each of these experiments based on numerical integration of the rate equations (computer simulation), the only things that differ for each experiment are the starting concentrations of the reactants and the definition of the output observables. Thus, all three experiments can easily be fit without concern over choosing the right equation, and they can be fit simultaneously using a single unifying model without approximations, *if the model is sufficient*. If the model is wrong, it can easily be modified. If the model gives a good fit, it is sufficient to account for the data. We can then use this model with computer simulation to design new experiments to further test the model.

Throughout the text, we focus on developing an understanding of the principles of kinetics and how they are applied to design informative experiments, and we interpret them rigorously based on computer simulation. When fitting various experiments using computer simulation, each experiment is based on the same model but with different experimental details and output functions, as summarized schematically in Figure 1.9.

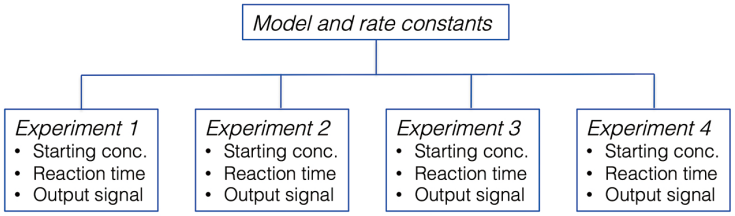

Figure 1.9 *Fitting data from multiple experiments with a single model.* This diagram illustrates the organizational hierarchy in *KinTek Explorer*. A single model is referenced in fitting multiple experiments which differ only in their starting concentrations, timescale of measurement, and output observable function. Experiments can include time-dependent reactions, equilibrium titrations, time-resolved spectra and other protocols.

This facilitates global data fitting. Figure 1.10 illustrates several examples of fitting data using equations, in contrast to fitting by simulation. The traditional process of data fitting involves deriving equations from a given model, then fitting the time dependence of the reaction to extract observed decay rates (eigenvalues) that reflect the underlying rate constants.[102] In a second step, the concentration dependence of the observed rates can be analyzed in a secondary plot. Thus, attempts to extract the primary kinetic parameters require application of yet another equation derived from the model.

As described in Chapter 5, steady-state data (Figure 1.10A) are first fit to a straight line to estimate the initial velocity, which is then plotted versus substrate concentration (Figure 1.10B) and fit to a hyperbola to get V_{max} and K_m. In this example, we then need to divide V_{max} by the extinction coefficient and enzyme concentration to get k_{cat}. Alternatively, we can fit data globally by simulation by simply entering the model, the starting concentrations of enzyme and substrate, and a mathematical description of what is being measured–in this case, absorbance due to the product with a known extinction coefficient (ε).

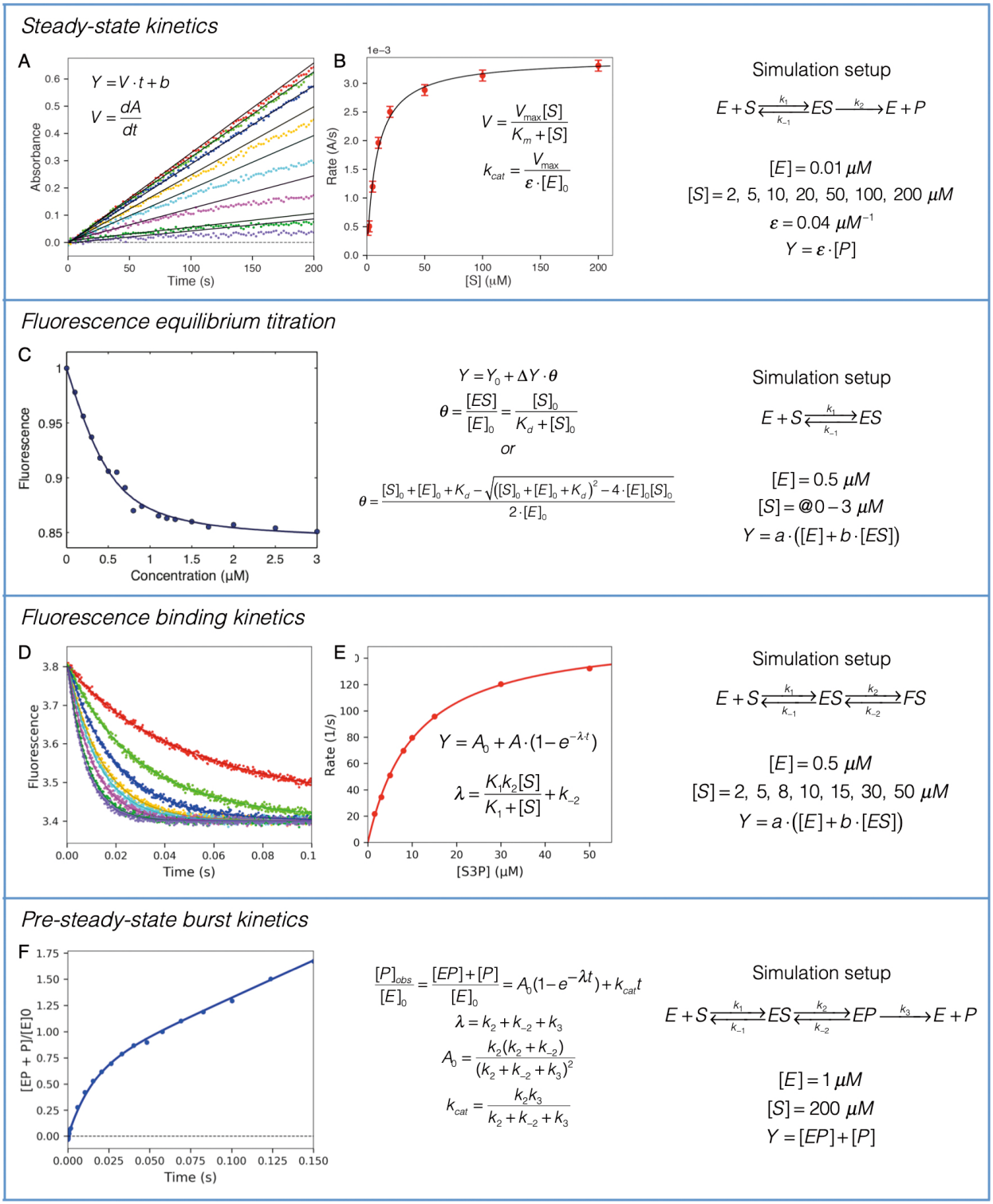

Figure 1.10 *Fitting data using equations versus by simulation.* On the left side we show examples data and the equations needed to fit each experiment. In the righthand column we show the setup to fit the data by simulation, without approximations and without concerns over choosing the right equation. To fit data by simulation, you only need a model, a set of starting concentrations, and a definition of what is being measured—then all experiments can be fit simultaneously.

In globally fitting data by simulation, we use a model to derive sets of *rate constants* based on numerical integration of rate equations (computer simulation), bypassing the tedious and error-prone approximations needed

to derive equations and fit data to arbitrary constants. Moreover, experiments are simulated exactly as performed, including the starting conditions, so that errors in interpretation are avoided.

Serious mistakes often result from using the wrong equation. For example, in Figure 1.10C, an equilibrium titration should be fit using a quadratic equation; use of a hyperbola gives the wrong answer, often by orders of magnitude, as explained in Chapter 3. Fitting the time dependence of a fluorescence change, shown in Figure 1.10D, first requires fitting by using a single exponential function (Chapter 8). The observed rate is then plotted versus substrate concentration and fit to a hyperbola to estimate the rate constants (Figure 1.10E). Often the data require a double exponential function, leading to more complex and error-prone analysis. Finally, a simple pre-steady-state burst experiment (Chapter 10) shown in Figure 1.10F can be fit to an exponential plus a linear phase, but the equations for the amplitude and rate are complex functions of intrinsic rate constants. In each of these cases, fitting by simulation can be accomplished simply by entering the model, starting concentrations of reactants, and a mathematical definition of what is being measured. Because of this simplicity, all of these experiments can be fit simultaneously by simulation based on a single unifying model.

Although we no longer rely on equations for rigorous data fitting, it is still important to understand the derivations of equations for simple reaction kinetics. The equations and concentration dependence of observed rates reveal the underlying mechanisms, which can then be entered into simulation software for more rigorous fitting. In addition, understanding the equations underlying the reaction kinetics helps to define the information content of the data and the conditions for performing each experiment. For example, Michaelis and Menten recognized that steady-state initial velocity measurements can be used to define the kinetics of enzyme catalysis even though their ultimate goal was to account for the full time course.[153,103] Moreover, understanding the underlying kinetics helps to design more direct measurements on a shorter time scale. Increasing the enzyme concentration and shortening the time scale of observation brings us into the realm of pre-steady-state kinetics, allowing observation of the reactions occurring at the active site of the enzyme, but the design of these experiments requires a knowledge of k_{cat} and K_m values. In each case the derivation of equations helps to define the conditions for the measurement, and analysis of the concentration dependence of the observable rates helps to suggest the reaction pathway. With this information in hand, the data can then be fit based on computer simulation to obtain the most accurate fit to the data. The use of equations in data fitting as a prelude to full simulation-based global data fitting is clearer in the context of more complex models, as described in Chapter 9.

Throughout this book, and especially in the latter chapters, we illustrate the successful application of global data fitting methods to different enzymes. This is based on the belief that teaching is more effective when abstract theory is applied to real-life examples. In many cases, the exam-

ples are taken from the author's research, where his firsthand experience affords insights into the challenges in solving the mechanism of a new enzyme. Each example illustrates that globally fitting data by simulation is far superior to traditional data fitting methods using equations. Not only is global fitting more accurate; often we see that the whole is greater than the sum of the parts. In fitting multiple experiments globally, we extract more information from the data than can be obtained by the piecemeal fitting of individual experiments to different equations.

1.8 Summary

Here we provide an introduction to kinetics, the definition of rate constants according to a model, and the means to define a mechanism based on measurements of the rate of reaction under a variety of conditions. This chapter serves as an overview of things to come so that as we dive deeper into the kinetics of individual reactions and different methods of measurement, you can keep in perspective why we are making a given measurement, where we are going, and how it all fits together. Our ultimate goal is to define a mechanism of reaction based on quantitative analysis to evaluate whether a given model meets the criteria of being both necessary and sufficient to account for the data. This quantitive analysis sets a high standard for evaluation of a proposed model.

1.8.1 Bullet point summary

- Models are important in biochemistry because they frame the questions to be addressed and provide a quantitive basis for fitting data to evaluate the validity of the model and predict new experiments.
- The term "rate constant" is only used to refer to a *true rate constant*, as illustrated by a lowercase k over an arrow in a pathway: $A \xrightarrow{k} B$.
- Equations can be solved for simple models according to the experiment, and these are valuable for preliminary data fitting to estimate parameters and suggest the underlying model.
- Simplifying approximations are required to solve most equations, which limits the range over which experiments can be interpreted to satisfy the approximations.
- Data fitting using exponential functions provides estimates of the net decay rate (eigenvalue). Eigenvalues are usually complex functions of rate constants and concentrations—they are not "observed rate constants."
- Various experiments differ only by the starting concentrations of reactants and the nature of the measured signal (Figure 1.10). This information is entered into *KinTek Explorer* software to model kinetics and fit data, allowing multiple experiments to be fit simultaneously using a single, unified model, and thereby transcending the need for a different equation to fit each experiment.