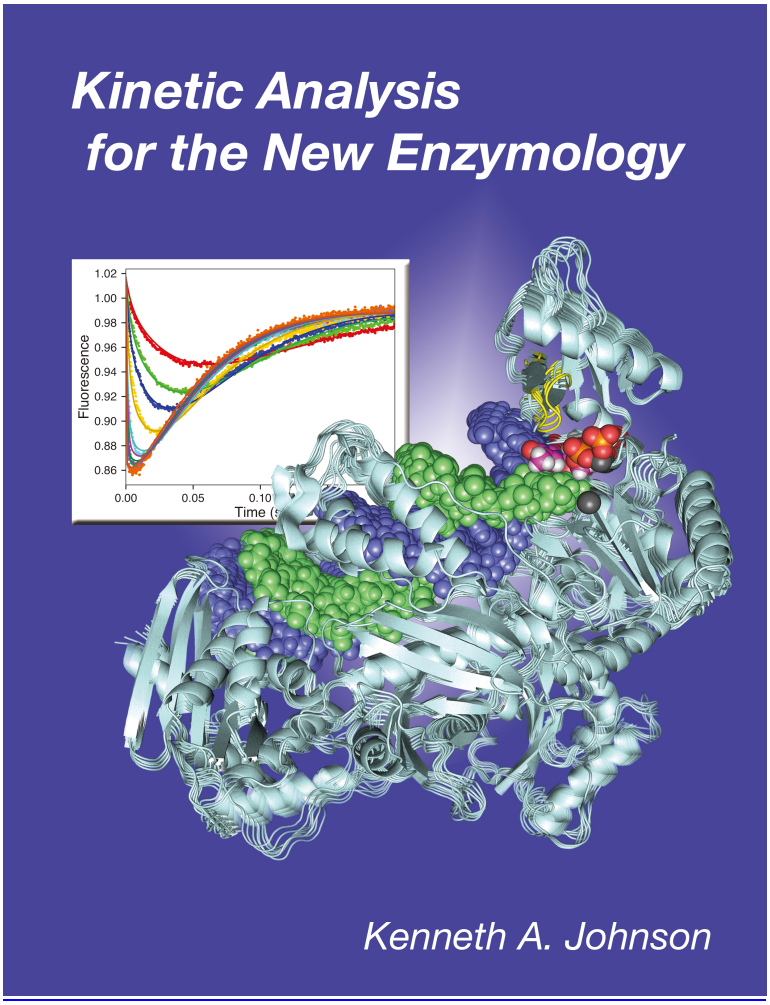
15th New Enzymology Kinetics Workshop

Kenneth A. Johnson
Austin, Texas
9-12 January 2024



This 480 page book is provided to participants.

15TH NEW ENZYMOLOGY KINETICS WORKSHOP

9-12 January 2024
Hyatt Regency on Lady Bird Lake
Austin, Texas

Tuesday 9 January	Foothills-2 (17th floor)
8:00 – 8:30 am	Registration
8:30 – 9:30 am	1. Introduction - 110 years of enzyme kinetics, 1913-2023
9:30– 10:00 am	*Refreshment Break*
10:00 – 11:30 am	2. Computer simulation I: Introduction to KinTek Explorer
11:30 – 1:30 pm	*Lunch*
1:30 – 3:00 pm	3. Ligand binding kinetics and modeling
3:00 – 3:30 pm	*Refreshment Break:*
3:30 – 5:00 pm	4. Ligand binding equilibria and data fitting by nonlinear regression
5:00 – 6:00 pm	Small Group Discussion: Computer Simulation Tutorial
Wednesday 10 January	Foothills-2 (17th floor)
8:00 – 9:30 am	5. Steady state kinetics and the meaning of k_{cat}, K_m and k_{cat}/K_m
9:30 – 10:00 am	*Refreshment Break:*
10:00 – 11:30 am	6. Kinetics of multi-step reactions
11:30 – 1:30 pm	*Lunch*
1:30 – 3:00 pm	7. Observing the chemical reaction at the enzyme active site
3:00 – 3:30 pm	*Refreshment Break:*
3:30 – 5:00 pm	8. Single-turnover kinetic studies and detection of intermediates
5:00 – 6:00 pm	Small Group Discussion: Kinetic Simulation Hands-on Tutorial
Thursday 11 January	Foothills-2 (17th floor)
8:00 – 9:30 am	9. Problem solving exercises I
9:30 – 10:00 am	*Refreshment Break:*
10:00 – 11:30 am	10. Interpreting stopped-flow signals: Induced-fit in enzyme specificity
11:30 – 1:30 pm	*Lunch*
1:30 – 3:00 pm	11. Computer simulation II: Advanced data fitting
3:00 – 3:30 pm	*Refreshment Break:*
3:30 – 5:00 pm	12. Kinetic analysis of slow binding inhibitors
5:00 – 6:00 pm	Small Group Discussion: Individual Question & Answer period
Friday 12 January	Foothills-2 (17th floor)
8:00 – 9:30 am	13. Problem solving exercises II
9:30 – 10:00 am	*Refreshment Break C*
10:00 – 11:30 am	14. pH and isotope effects: Lessons from DHFR
11:30 – 1:30 pm	*Lunch*
1:30 – 3:00 pm	15. Single molecule kinetics
3:00 – 3:30 pm	*Refreshment Break:*
3:30 – 5:00 pm	16. Global Fitting: Putting it all together
5:00 – 6:00 pm	Group Discussion: Individual question and answer period
7:00 – 9:00 am	Graduation Celebration Dinner: Foothills-2 (17th floor)

Overview: Modern kinetic methods coupled with high resolution structural data provide powerful tools to establish reaction mechanisms. In this four-day workshop, modern kinetic analysis will be described with numerous examples of the application of advanced kinetic methods to study proteins and nucleic acids. The workshop will focus on developing the path from experimental design to data collection and analysis to yield new mechanistic insights.

KinTek Global Kinetic Explorer, a dynamic and power computer simulation program, will form a cornerstone of the course in illustrating important concepts and to develop a better intuitive understanding of observable reaction kinetics. A major emphasis will be on understanding and extracting the full *information content* of kinetic and equilibrium binding data. A copy of Dr. Johnson's book will be given to each participant: "*Kinetic Analysis for the New Enzymology—using computer simulation to learn kinetics and solve mechanisms*."

The following topics will be discussed.

1. *Mechanism-based data fitting*. The course will focus on the use of computer simulation based on numerical integration of rate equations obtained from a model entered by the user. The primary goal of the workshop will be to help you to become proficient in modeling and fitting data using KinTek Explorer software so that you can tackle any problem.
2. *Steady state kinetics*. The information content of steady state kinetic measurements will be described in discussing the meaning of the kinetic constants, what they tell about a reaction mechanism and what they do not reveal.
3. *Introduction to transient kinetic methods.* The need for transient kinetic methods will be described to provide mechanistic information that can be interpreted directly to define reactions occurring at the active sites of enzymes or nucleic acids.
4. *Fundamental principles of reaction kinetics*. The basic principles of reaction rate measurement will be described. The simple math behind exponential reaction kinetics will be presented to reveal the information content of kinetic data.
5. *Data fitting principles and practice*. The use of computer simulation and nonlinear regression in data fitting will be described. Equations will be presented for general use in data fitting and the meaning of the kinetic parameters will be described. We will show how mechanism-based data fitting overcomes the many limitations of equation-based fitting.
6. *Kinetics of ligand binding*. We will begin a discussion of reaction kinetics with the binding of a ligand to a protein or nucleic acid to emphasize the importance of analysis of the concentration dependence of the rate. We will also discuss the relationship between the binding rate constant and the steady state kinetic parameter k_{cat}/K_m and the principles governing enzyme efficiency and specificity.
7. *Kinetics of multi-step reactions*. The kinetics of two-step reactions will be described under different scenarios. The principles that govern the design of experiments and the modeling of data to distinguish alternatives will be discussed.
8. *Kinetics of ligand dissociation*. The kinetics of ligand dissociation in competition experiments will be explored. Examples include the use of protein fluorescence and of fluorescently labeled substrates to measure release of an enzyme substrate from either protein or RNA enzymes.
9. *Analysis of chemical-quench-flow data*. Chemical-quench-flow experiments are often more difficult to perform, but easier to interpret because the direct measurement of the conversion of substrate to product eliminates ambiguities in the possible

interpretations. These experiments anchor the interpretation of fluorescence experiments in globally fitting data.

10. *Single turnover kinetic studies*. The best experiments to look for enzyme intermediates are based upon studies of the conversion of substrate to product with enzyme in excess over limiting substrate. The design criteria for such experiments and their interpretation will be described with examples from several enzymes.

11. *Interpretation of stopped-flow signals*. Fluorescence signals are often difficult the origins of the fluorescence change may not be known. These problems are solved by globally fitting data defining the optical changes relative to the individual species in a reaction sequence. We will also describe singular value decomposition methods to analyze time-dependent spectral changes.

12. *Kinetics of slow binding inhibitors*. Many potent enzyme inhibitors bind so tightly that at concentrations near their K_d the rates of binding are quite slow. Although analysis of slow, tight binding inhibitors is a traditional steady state kinetic problem, the data can best be analyzed by computer simulation.

13. *Single molecule kinetic methods*. Observation of single molecules presents several advantages over measurements of ensembles of molecules in bulk solutions but is not without its serious limitations. Our discussion will focus on the relationship between single molecule and ensemble measurements and how the two methods can be used together to gain new mechanistic information.

14. *Global data fitting methods*. We will show how global data fitting based on computer simulation provides the most rigorous method to extract mechanistic information. This analysis reveals a complete pathway in ways that cannot be obtained by piecemeal equation-based data fitting. In global data fitting we show that the whole is greater than the sum of the parts.