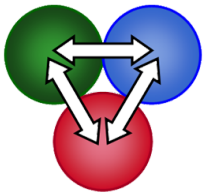

14th New Enzymology Kinetics Workshop

Kenneth A. Johnson
Austin, Texas
2-6 January 2020

14TH NEW ENZYMOLOGY KINETICS WORKSHOP

2-6 January 2020
Hyatt n Town Lake
Austin, Texas

Thursday 2 January

6:00 – 7:00 pm	Registration: Reception area
7:00 – 9:00 pm	Welcome Reception

Friday 3 January :

8:00 – 9:30 am	1. Introduction - 100 years of enzyme kinetics, 1913-2013
9:30 – 10:00 am	*Refreshment Break*
10:00 – 11:30 am	2. Computer simulation I: Introduction to KinTek Explorer
11:30 – 1:00 pm	*Lunch*
1:00 – 2:30 pm	3. Steady state kinetics and the meaning of k_{cat}, K_m and k_{cat}/K_m
2:30 – 3:00 pm	*Refreshment Break:*
3:00 – 4:30 pm	4. Ligand binding equilibria and data fitting by nonlinear regression
4:30 – 5:30 pm	Discussion: Nonlinear Regression & Computer Simulation

Saturday 4 January

8:00 – 9:30 am	5. Transient kinetic methods and ligand binding kinetics
9:30 – 10:00 am	*Refreshment Break:*
10:00 – 11:30 am	6. Kinetics of multi-step reactions
11:30 – 1:00 pm	*Lunch*
1:00 – 2:30 pm	7. Chemical quench-flow data and pre-steady state burst
2:30 – 3:00 pm	*Refreshment Break:*
3:00 – 4:30 pm	8. Single-turnover kinetic studies and detection of intermediates
4:30 – 5:30 pm	Small Group Discussion: Kinetic Simulation Hands-on Tutorial

Sunday 5 January

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 – 1:00 pm	*Lunch*
1:00 – 2:30 pm	11. Computer simulation II: Advanced data fitting
2:30 – 3:00 pm	*Refreshment Break:*
3:00 – 4:30 pm	12. Kinetic analysis of slow binding inhibitors
4:30 – 5:30 pm	Small Group: Individual Question & Answer period

Monday 6 January

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 – 1:00 pm	*Lunch*
1:00 – 2:30 pm	15. Single molecule kinetics
2:30 – 3:00 pm	*Refreshment Break:*
3:00 – 4:30 pm	16. Global Fitting: Putting it all together
4:30 – 5:30 pm	Individual question and answer period
8:00 – 9:30 am	Graduation Celebration Dinner

Overview: Modern kinetic methods coupled with high resolution structural data provide a powerful tool to establish reaction mechanisms. In this four-day workshop, modern kinetic analysis will be described with numerous examples of the application of stopped-flow and chemical-quench-flow 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. Computer simulation will be used to develop a better intuitive understanding of observable reaction kinetics dependent upon different underlying mechanisms. *KinTek Global Kinetic Explorer*, a dynamic and power simulation program, will form a cornerstone of the course in illustrating important concepts. The following topics will be discussed.

1. Steady state kinetics. The information content of steady state kinetic measurements will be described by presenting the meaning of the kinetic constants, what they tell about a reaction mechanism and what they do not reveal. Understanding the limitations of the information available from steady state kinetics highlights the need for techniques to examine directly the reactions occurring at the active sites of enzymes.

2. Introduction to rapid mixing methods. The need for rapid mixing methods will be described relative to the limitations in the information content of steady state kinetic parameters and the need to provide mechanistic information that can be interpreted directly relative to reactions occurring at the active sites of enzymes or nucleic acids. Basic principles of mixing methods will be described to highlight the potential and limitations of the methods in principle and in practice. Stopped-flow, chemical quench-flow, and continuous flow mixing methods will be described.

3. Fundamental principles of reaction kinetics. The basic principles of reaction rate measurement will be described including the difference between initial rate and full time course rate measurements. The simple math behind exponential reaction kinetics will be presented as a prelude to understanding the equations used in data fitting for more complex reaction pathways.

4. Data fitting principles and practice. The use of non-linear regression and computer simulation in data fitting will be discussed. Equations will be presented for general use in data fitting and the meaning of the kinetic parameters will be described. Older methods of data fitting relying upon linearized equations will be described along with a list of reasons why these methods should no longer be used.

5. Kinetics of ligand binding. We will begin a discussion of reaction kinetics with the binding of a ligand to a protein or nucleic acid. The basis for the reaction conditions needed to achieve pseudo-first-order kinetics and the importance of analysis of the concentration dependence of the rate will be discussed. Examples will include the binding of fluorescently labeled oligonucleotides to ribozymes, ATP to motor proteins and substrates to enzymes. We will also discuss the relationship between the binding rate and the steady state kinetic parameter k_{cat}/K_m and the principles governing enzyme efficiency and specificity.

6. Kinetics of multi-step reactions. The kinetics of two-step reactions will be described under different scenarios. For example, if the first step is a rapid equilibrium relative to the second step, the equations describing the process and the results of data fitting are simplified. Alternatively, there are circumstances whereby all four rate constants governing a two step binding reaction can be obtained from the concentration dependence of the observed rates. The principles that govern the design of experiments and the modeling of data to distinguish alternatives will be discussed.

7. 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. We will address the question of circumstances under which the ratio of the rate of binding divided by the rate of dissociation can be used to define the equilibrium constant for binding.

8. Analysis of chemical-quench-flow data. Chemical-quench-flow experiments are often more difficult to perform, but usually more easy to interpret because of the absolute amplitude information and the direct measurement of the conversion of substrate to product eliminate ambiguities in the possible interpretations. We will discuss the design and execution of chemical-quench-flow studies, including the basis for pre-steady-state burst experiments and substrate trapping experiments.

9. 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 EPSP synthase, which will also serve to illustrate the pitfalls of interpreting structural data in the absence of kinetic data.

10. Interpretation of stopped-flow signals. The particular difficulties of interpretation of signals in the stopped-flow will be described by outlining the possible origins of the optical changes observed relative to the individual species in a reaction sequence. Particular difficulties in the interpretation of fluorescence data will be highlighted. We will also describe singular value decomposition methods to analyze time-dependent spectral changes.

11. 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 on the basis of burst kinetics. We will present the rationale and equations to accurately analyze the time dependence of slow onset inhibition.

12. 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.

13. Global fitting methods. Methods for fitting data directly to kinetic models by computer simulation will be discussed. The difficulties and challenges as well as the benefits of this modern approach will be presented. Examples involving the fitting of real and simulated data will be included. In addition, we will explain conditions under which conventional data fitting methods fail, requiring the use of computer simulation.