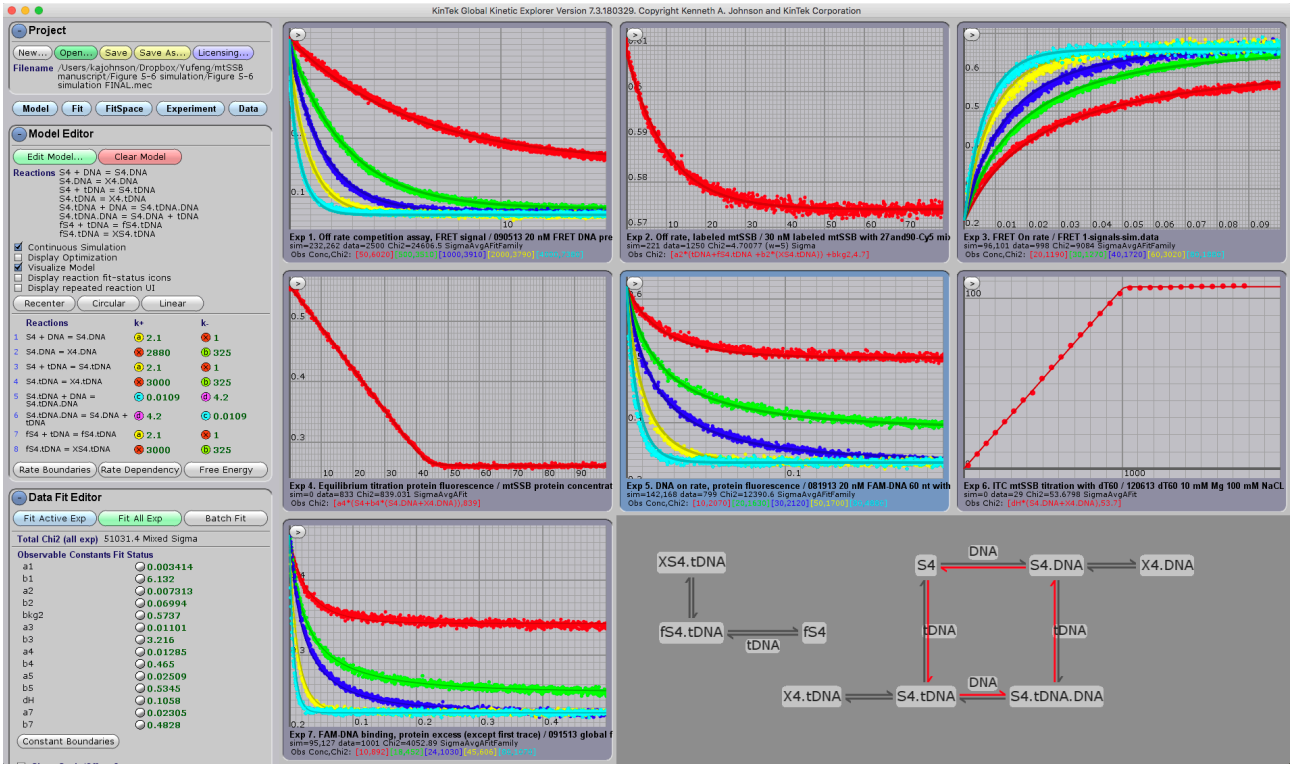
13TH NEW ENZYMOLOGY KINETICS WORKSHOP

OFFERED BY

KENNETH A. JOHNSON, PH.D.

26 May – 30 May 2019
Hotel International BRNO, Czech Republic



This figure shows the simultaneous fitting six experiments to define the kinetics and equilibria for the binding of ssDNA to the human mitochondrial ssDNA binding protein. This work was featured as an Editors' Pick in the Journal of Biological Chemistry, July 2017.

IN 30+ HOURS OF INTENSE INSTRUCTION YOU WILL LEARN TO:

- Understand the dynamics of enzyme reactions
- Plan and execute informative kinetic experiments
- Fit kinetic data to extract mechanistic information
- Understand the information content of kinetic data
- Use simulation to predict results
- Fit data directly to your model
- Fit pharmacokinetic/dynamic and SPR data
- Globally fit multiple experiments to a single unifying model

For more Information or to download *KinTek Explorer* software, go to: www.kintekexplorer.com

13TH NEW ENZYMOLOGY KINETICS WORKSHOP SCHEDULE

26 May – 30 May 2019
Hotel International Brno
Brno, Czech Republic

Sunday 26 May 2019: Reception, 7:00–9:00 p.m., Hotel International Brno

Monday, 27 May 2019

7:30 – 8:45 a.m.	*Breakfast*
9:00 – 10:30 a.m.	1. Introduction
10:30 – 10:45 a.m.	*Refreshment Break*
10:45 – 12:00 a.m.	2. Steady state kinetics and the meaning of k_{cat} and K_m
12:00 – 1:30 p.m.	*Lunch*
1:30 – 3:00 p.m.	3. Ligand binding equilibria and nonlinear regression data fitting
3:00 – 3:15 p.m.	*Refreshment Break*
3:15 – 4:45 p.m.	4. Computer simulation tutorial I: Introduction
4:45 – 6:00 p.m.	Small group discussion: Nonlinear regression & simulation

Tuesday, 28 May 2019

7:30 – 8:45 a.m.	*Buffet breakfast*
9:00 – 10:30 a.m.	5. Transient kinetic methods and simple ligand binding
10:30 – 10:45 a.m.	*Refreshment Break*
10:45 – 12:00 a.m.	6. Kinetics of multi-step reactions
12:00 – 1:30 p.m.	*Lunch*
1:30 – 3:00 p.m.	7. Chemical quench-flow data and pre-steady state burst
3:00 – 3:15 p.m.	*Refreshment Break*
3:15 – 4:45 p.m.	8. Single-turnover kinetic studies and detection of intermediates
4:45 – 6:00 p.m.	Small group discussion: Kinetic simulation hands-on tutorial

Wednesday, 29 May 2019

7:30 – 8:45 a.m.	*Buffet breakfast*
9:00 – 10:30 a.m.	9. Problem solving 1: Computer simulation exercises
10:30 – 10:45 a.m.	*Refreshment Break*
10:45 – 12:00 a.m.	10. Interpreting fluorescence signals
12:00 – 1:30 p.m.	*Lunch*
1:30 – 3:00 p.m.	11. Computer simulation II: data fitting
3:00 – 3:15 p.m.	*Refreshment Break*
3:15 – 4:45 p.m.	12. Kinetics of slow binding inhibitors
4:45 – 6:00 p.m.	Small Group: Individual question & answer period

Thursday, 30 May 2019

7:30 – 8:45 a.m.	*Buffet breakfast*
9:00 – 10:30 a.m.	13. Problem solving session II: Data analysis
10:30 – 10:45 a.m.	*Refreshment Break*
10:45 – 12:00 a.m.	12. Single molecule kinetics
12:00 – 1:30 p.m.	*Lunch*
1:30 – 3:00 p.m.	13. Modeling SPR and pharmacokinetics.
3:00 – 3:15 p.m.	*Refreshment Break*
3:15 – 4:45 p.m.	14. Global data fitting: putting it all together
4:45 – 6:00 p.m.	Individual question & answer period
6:30 – 9:00 p.m.	**Graduation Celebration Dinner**

Overview of the New Enzymology Kinetics Workshop: Modern kinetic methods coupled with high resolution structural data provide a powerful tool to establish reaction mechanisms. In this intensive four-day course taught by Dr. Kenneth A. Johnson, modern kinetic analysis will be described with numerous examples of the application of kinetic and equilibrium methods to study proteins and nucleic acids. The course will focus on developing the path from experimental design to data collection and analysis to yield new mechanistic insights. In addition, tutorials on the use of computer simulation will develop a better intuitive understanding of reaction kinetics. Hands on problem solving will facilitate integration of the course material. A central feature of the course is the use of *KinTek Explorer* software to learn kinetics and rigorously fit data. Participants will receive a temporary license for the software (for Mac of Windows) to use during the course.

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. We will also cover the kinetics of enzyme inhibition and enzyme activation. We will also discuss the relationships between the binding rate and the steady state kinetic parameter k_{cat}/K_m and the principles governing enzyme efficiency and specificity.
2. Equilibrium binding measurements. An overview of the methods and importance of equilibrium binding measurements will be presented. Equations for data fitting will be discussed with an emphasis on choosing the right equation and fitting by nonlinear regression. Moreover, we will reveal novel methods for simulating equilibrium titrations so that kinetic and equilibrium data can be fitted simultaneously.
3. Introduction to rapid mixing methods. The basis for rapid mixing methods will be described to provide mechanistic information that can be interpreted directly relative to reactions occurring at the active sites of enzymes. Basic principles of mixing methods will be described to highlight the potential and limitations of the methods in principle and in practice. Stopped-flow and chemical quench-flow will be described. We can also model temperature-jump experiments as part of a comprehensive global data fitting.
4. 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. These principles lay the foundation for understanding data fitting based upon numerical integration of the rate equations (simulation) with no simplifying approximations.
5. 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. Moreover, fitting to equations reveals patterns in the data that help to develop a model and serve as a basis for global data fitting based upon computer simulation. Throughout the course, we will emphasize the developing of intuition that allows for understanding more complex reaction schemes.
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. 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.

7. Kinetics of multi-step reactions. The kinetics of two-step reactions will be described under different scenarios including circumstances under which 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.
8. Analysis of chemical-quench-flow data. Chemical-quench-flow experiments are often more difficult to perform, but more easy to interpret because of the absolute amplitude information and the direct measurement of the conversion of substrate to product eliminates 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. Interpretation of fluorescence 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 and solutions to these problems will be presented based upon computer simulation and global data fitting.
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 EPSP synthase, which will also serve to highlight the pitfalls of interpreting structural data in the absence of kinetic data.
11. Global fitting methods. The new *KinTek Explorer* software will be introduced. This powerful, dynamic software allows new insights into understanding kinetics, planning experiments and fitting data. Methods for fitting data directly to kinetic models by computer simulation will be presented. The difficulties and challenges as well as the benefits of this modern approach will be discussed, including examples involving the fitting of real and simulated data. In addition, we will explain conditions under which conventional data fitting methods fail, requiring the use of computer simulation.
12. Single-molecule kinetics. Rationale, methods and analysis for single molecule kinetic studies will be described. The unique capabilities as well as the challenges in examining kinetics of single molecules will be presented, based upon several outstanding examples including HIV reverse transcriptase, DNA sequencing, RNA folding and the kinesin ATPase motor protein. New methods of fitting single molecule kinetic data will be presented.
13. SVD analysis of time or concentration-dependent spectra. Singular value decomposition (SVD) allows resolution of the time dependence and spectra of individual species in a reaction. This method will be introduced and explained, with the simplicity of the powerful *KinTek Explorer* software.
14. SPR kinetics. Although there are still limitations to interpretation of SPR (Surface Plasmon Resonance) measurements due to surface and mass transfer effects, *KinTek Explorer* provides a method to afford rigorous analysis of SPR data. In this workshop, we will teach some of the shortcuts to make modeling SPR data easy and fast and what to watch out for in designing and interpreting results.
15. Pharmacokinetics/dynamcis. *KinTek Explorer* provides the most powerful platform for modeling and fitting pharmacokinetic/dynamic data. The user can easily enter any model. Compartments are modeled as species and rate constants contain a term for volume of the compartment. In the workshop, we will show how easy it is to rigorously fit pharmacokinetic/dynamic data and predict phenomena that you may not be able to observe directly.

Prospectus

Participants must bring their personal computers in order to develop expertise in the use KinTek Explorer simulate kinetics. This powerful new tool provides hands-on experience in relating observable kinetic data to underlying models, enabling researchers to pose questions such as: What would the data look like for a given model? Can a proposed experiment distinguish alternative models? Which rate constants are determined by these data? Moreover, KinTek Explorer Software will be used to illustrate the fitting of data by nonlinear regression directly to a model. Fitting data by simulation directly to a model represents a significant paradigm shift that requires the development of some intuition and insight. In this short course we will work to develop that intuition and teach the skills needed for rigorous data analysis. Be sure to familiarize yourself with KinTek Explorer in advance of coming by downloading the FREE student version (by running the unlicensed version) and manual if you have not already. Data sets are provided for your use in practice.

Register Now

- **We limit registration to provide personalized instruction.**
- **Register before 1 March 2019 to get reduced registration rates!**
- **Make your hotel reservation now to reserve your seat and ensure reduced hotel room rates.**

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Intensive Kinetics Short Course 2019 Registration Form

26 May - 30 May 2019 ◆ Brno, Czech Republic

Please fill out this form and mail or FAX (512) 899-2116, for each person.

Please be especially careful in entering your email address

Name, Title _____

Company/University _____

Address 1 _____

Address 2 _____

City, State, Country, Zip _____

Phone: _____ Fax: _____

Email: _____

Circle One: Grad Student Post Doc Professor Research Scientist Industry

Attach your vita to this form.

Please describe briefly your area of research and what you would like to get out of the workshop.

Included in the registration fee: Course materials including a 120 page booklet on kinetics, opening reception, final banquet, and breakfast, morning and afternoon breaks, and lunch each day.

Payments in US Dollars may be made by check to: "KinTek Corporation / Kinetics Short Course" or by credit card authorization which will be processed by our sponsor, KinTek Corporation.

Credit Card Authorization (Visa or MasterCard ONLY) Please circle appropriate price.

	Industry/Private Sector	Academic/Govt	Grad Student
Before 1 March 2019	$2980.00	$1490.00	$995.00
After 1 March 2019	$3480.00	$1750.00	$1250.00

Card Number _____Exp. Date_____Security Code_____

Signature _____

***Cancellations must be submitted prior to 1 March 2019 to receive a full refund.**

Return completed order forms to:

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