AppliedPhotophysics

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Biochemical kinetics and reaction mechanisms

Biochemical reactions carried out by enzymes are fundamental to the metabolic processes of catabolism, and anabolism. Similarly the binding events and signal modifications that are carried out by signaling and receptors proteins are important in the control functions of an organism. Studies of the mechanisms of these important biochemical agents allows insights into how an organisms functions at the molecular level.

The kinetics of biochemical reactions involves the study of rates of chemical processes involved in many processes. Measurements of the rates of reactions under different experimental conditions (for instance pH, solvent, concentration and temperature) allow the construction of models, using software tools like Pro-K II, that describe the characteristics of a biochemical reaction. This model provides insights into the reaction mechanisms involved in the reaction. The most important mechanistic reactions can be broadly classed as binding events, and enzymatic catalysis.

Binding events, such a protein-ligand binding and release, are fundamental to all biochemistry, from signaling pathways to binding of reactants in enzymatic reactions. The use of stopped-flow spectrophotometer are particularly powerful tools to study the kinetics of binding reactions, using fluorescence and other optical probes. This can provide information about the mechanisms of binding, and the energies involved.

Enzymatic reactions catalyze the conversion of metabolites and are the agents that carry out the large variety of specific chemical reactions in biology. As with all chemical kinetics, understanding of the number and rate of reactions, and building of reactions models around this information can provide profound insights into the mechanism of actions at the chemical level for enzymatic reactions.

Stopped-flow spectrophotometers like the SX20 allow the following of reaction kinetics, initiated by the mixing of two (or more) reactants, from the millisecond time range onwards, using changes in various optical probes, like fluorescence, fluorescence anisotropy, absorbance and circular dichroism. This allows a very diverse range of mechanism of many different types of biochemical reactions to be studied in great depth. For a more in depth explanation of the stopped-flow method please read the tutorial.

Laser flash photolysis using the LKS.60 allows the reactions occurring in the nanosecond time range to be studied. Reactions are initiated by a very brief pulse of laser light. Then data is collected using a number of spectrometric techniques. This allows ultrafast reactions processes to be studied. For a more in depth explanation of the laser flash photolysis method please read the tutorial.

Relevant Binding Mechanisms References

Listed below are 5 selected recent references of studies of various binding and macromolecular interaction mechanisms using APL stopped-flow and laser flash photolysis systems. A complete searchable database with all references can be accessed by logging into the APL members area.

Authors	Title	Year	Keywords	Journal/Proceedings
Oleg V. Moskvin, Samuel Kaplan, Marie-Alda Gilles-Gonzalez, and Mark Gomelsky	Novel Heme-based Oxygen Sensor with a Revealing Evolutionary History [**Abstract**] [**URL**]	2007	Signal transduction, Oxygen Sensor, hemeprotein	J BIOL CHEM, 2007, Vol 282, Iss 39, pp 28740-28748
Celestine N Chi, Lisa Elfstrom, Yao Shi,	Reassessing a sparse energetic network within a single protein domain	2008	allostery, coupling energy, dynamics,	PNAS 2008 vol. 105 no. 12 pp 4679-4684

Engstrom, Per Jemth	[Abstract] [URL]		residues, PDZ domain	
Dina Grohmann, Dr., Valentina Corradi, Dr., Mira Elbasyouny , Annika Baude , Florian Horenkamp, Sandra D. Laufer , Fabrizio Manetti, Dr. , Maurizio Botta, Prof. , Tobias Restle, Prof.	Small Molecule Inhibitors Targeting HIV-1 Reverse Transcriptase Dimerization [Abstract] [URL]	2008	antiviral agents, dimerization, drug design , HIV reverse transcriptase	ChemBioChem Volume 9 Issue 6, Pages 916 - 922
Hannah S. Timsa and Jonathan Widom	Stopped-flow fluorescence resonance energy transfer for analysis of nucleosome dynamics [Abstract] [URL]	2007	Chromatin; Histones; LexA protein; DNA; Nucleosome reconstitution; FRET; Kinetics; Cy-dye labeling; Conformational dynamics; Site exposure	Methods Volume 41, Issue 3, March 2007, Pages 296-303
Yannick H. Ouellet, Richard Daigle, Patrick Lag□e, David Dantsker, Mario Milani, Martino Bolognesi, Joel M. Friedman, and Michel Guertin	Ligand Binding to Truncated Hemoglobin N from Mycobacterium tuberculosis Is Strongly Modulated by the Interplay between the Distal Heme Pocket Residues and Internal Water [Abstract] [URL]	2008	Nitric oxide, laser flash photolysis, hemoglobin, binding	J. Biol. Chem., Vol. 283, Issue 40, 27270-27278, October 3.

Relevant Enzyme Mechanisms References

Listed below are 5 selected recent references of studies a number of enzyme kinetic mechanisms using APL stopped-flow and laser flash photolysis systems. A complete searchable database with all references can be accessed by logging into the APL members area.

Authors	Title	Year	Keywords	Journal/Proceedings
Emile Bol, Nicolette J. Broers, and Wilfred R. Hagen	A steady-state and pre-steady-state kinetics study of the tungstoenzyme formaldehyde ferredoxin oxidoreductase from Pyrococcus furiosus [Abstract] [URL]	2008	Tungsten, Formaldehyde oxidoreductase, Pyrococcus furiosus, Pre-steady-state kinetics, Steady-state kinetics	J BIOL INORG CHEM, 2008, Vol 13, Iss 1, pp 75-84
Hothi, P.; Lee, M.; Cullis, P. M.; Leys, D.; Scrutton, N. S.	Catalysis by the Isolated Tryptophan Tryptophylquinone-Containing Subunit of Aromatic Amine Dehydrogenase Is Distinct from Native Enzyme and Synthetic Model Compounds and Allows Further Probing of TTQ Mechanism [Abstract] [URL]	2008	benzylamines, Aromatic Amine Dehydrogenase, TTQ Mechanism	BIOCHEMISTRY-USA, 2008, Vol 47, Iss 1, pp 183-194
Shiva Bhowmik, Geoff P. Horsman, Jeffrey T. Bolin, and Lindsay D. Eltis	The Molecular Basis for Inhibition of BphD, a C-C Bond Hydrolase Involved in Polychlorinated Biphenyls Degradation: LARGE 3-SUBSTITUENTS PREVENT TAUTOMERIZATION [Abstract] [URL]	2007	polychlorinated biphenyls, PCB, Bph, BphD, catalysis	J BIOL CHEM, 2007, Vol 282
William C. Cooper, Yi Jin, and Trevor M. Penning	Elucidation of a Complete Kinetic Mechanism for a Mammalian Hydroxysteroid Dehydrogenase (HSD) and Identification of All Enzyme Forms on the Reaction Coordinate: THE EXAMPLE OF RAT LIVER 3α-HSD (AKR1C9) [Abstract] [URL]	2007	Hydroxysteroid dehydrogenases, steroid biosynthesis, enzyme kinetics	J BIOL CHEM, 2007, Vol 282, Iss 46, pp 33484-33493
Derren J. Heyes, Michiyo Sakuma, and Nigel S. Scrutton	Laser Excitation Studies of the Product Release Steps in the Catalytic Cycle of the Light-driven Enzyme, Protochlorophyllide Oxidoreductase [Abstract] [URL]	2007	Protochlorophyllide Oxidoreductase, Light-driven Enzyme, Laser Excitation, enzyme catalysis	J BIOL CHEM, 2007, Vol 282, Iss 44, pp 32015-32020

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