Syllabus for Chemistry 5.50  Fall 2002 T-Th at 10-11:30, Room 1-150

This course will examine the details of how structure determines function for all major classes of enzymes. A tool box of methods will be presented to study any catalytic system. Methods that will be discussed include structure (determined by crystallographic and NMR methods), kinetics (steady state and presteady state methods to look for chemically and kinetically competent intermediates), isotope effects, a variety of exchange reactions (to look for intermediates), stereochemical methods, site directed mutagenesis, methods to replace natural with unnatural amino acids, mechanism based inhibitors, group selective reagents, and selection methods to establish substrate specificity. For each class of enzyme, a generic mechanism (or mechanisms) and an overview of their importance in metabolism will be presented. One enzyme from each class will then be discussed in detail using structure as a starting point. One or several new tools will be presented with the introduction of each new class of enzyme. Previous methods will be used to study each successive system to demonstrate the versatility of the methods. The goal is to provide you with general mechanistic insight about all metabolic reactions and to provide you with a tool box to study any new reaction that you may encounter.

Date        Topic

Sept. 5, 10, 12  Rate Acceleration and substrate specificity: use of binding energy in catalysis, use of acid/base and nucleophilic catalysis; transition state vs ground state (preorganization) controversy; chorismate mutases, serine proteases, OMP decarboxylases will be examined.


Sept. 17, 19, 24, 26  Proteases: Serine proteases will be used as prototypes to describe a number of methods to unravel the details of catalysis: steady state and presteady state kinetics, determination of the basis of substrate specificity, methods to establish the chemical and kinetic competence of covalent (acyl enzyme) intermediates, use of mechanism based inhibitors.


Oct. 1, 3  Phosphoryl Transfer Reactions: Kinases and phosphatases play a major regulatory role in biology. The chemistry at phosphorous vs carbon will be discussed. The chemistry of ATP will be presented. Tyrosine phosphatases will be used as the prototype. The new methods introduced will be stereochemistry, pH rate profiles and site directed mutagenesis to study groups involved in catalysis. If time permits, experimental approaches to determine the role of metal ions in catalysis will be discussed. The issues discussed are directly relevant to RNA catalyzed reactions.


Oct. 8, 10, 17, 22  Carbon-carbon Bond Formation: The generic mechanisms for Claisen and Aldol condensations and prenyl transfer reactions will be discussed. The use of kinetic isotope effects and positional isotope exchange methods will be introduced. The use of isotopes
(chiral methyls) to study stereochemical questions will be presented. Citrate synthase, D-2-deoxyribose-5-P aldolase and farnesyl pyrophosphate synthase will be examined in depth.


Oct. 24, 29 **Isomerases:** The general strategy for enzyme-catalyzed abstraction of α-protons of carboxylic acids will be discussed. Mandelate Racemase and enolase will be used as prototypes. The use of exchange reactions to look for intermediates will be presented. The superfamily issue and approaches to identification of superfamilies will be presented. The issue of whether the function of an unknown unknown open reading frame in a new genome can be assigned based on knowledge of active site chemistry will be discussed?

**Assignment:** Babbitt and Gerlt, "Understanding enzyme superfamilies. Chemistry as the fundamental determinant in the evolution of new catalytic activities. J. Biol Chem 1997 272, 30591-4.

Oct. 31, Nov. 5 **Pyridoxal Phosphate Requiring Enzymes:** General principles of this cofactor chemistry will be presented, as will the diversity of structures of PLP enzymes. D and L Aspartate Aminotransferase will be uses as prototypes. Use of unnatural amino acids and chemical rescue methods will be introduced.

**Assignment:** Toney and Kirsch (1989) Science 243, 1485-1488 Direct Bronsted Analysis of the Restoration of Activity to a Mutant Enzyme by Exogenous Amines"

Nov. 7, 12, 14, 19 **Oxidation/Reduction Cofactors NAD, Flavins, Pyrolloquinoline quinones and analogs:** Generic mechanisms for each redox cofactor will be presented and an introduction to one electron vs two electron chemistry and how protein environments modulate redox potentials will be addressed. Alcohol Dehydrogenase (NAD), p-Hydroxybenzoate Hydroxylase (FAD) and Plasma amine oxidase will be used as prototypes. The possibility of tunneling in enzymatic "H" transfer reactions will be presented. The importance of the barrier width rather than the barrier height will be discussed.


Nov. 21, 26. Dec. 3 **Introduction to Enzymes that use one electron chemistry [protein radicals (tyrosyl, glycyl and thiyl radicals) and cofactor radicals (PLP, thiamin and flavins)].** Steady state EPR spectroscopy to look at transient radical intermediates will be presented. Lysine amino mutase will be examined in detail. Pyruvate: Ferredoxin Oxidoreductase in comparison with Pyruvate Dehydrogenase and the one electron vs two electron roles of thiamin will be presented.

Dec. 5, 10  **Adenosylcobalamin dependent Chemistry**: Heterolytic and hemolytic chemistry of cobalamin cofactors. Diol dehydratase will be used as prototypes. Use of stopped flow spectroscopy and rapid freeze quench EPR spectroscopy, isotope effects, previously used methods, will be reused.


Dec 12  **Overview of unusual metallo cofactors**: New frontiers in protein structure and function.

Choices of enzymes for final examine presentation: Uracil Glycosylases (Tainer); Histidine Ammonia Lyase (Retey); Glycosidases (Whithers); Amidotransferases (Smith, J. and Holden, H.); Terpene cyclases (Noel); ATP grasp superfamily (Knox and Walsh); DNA polymerases (Ellenberger, Benkovic and Johnson); Caspases (proteases involved in apoptosis); Pyruvate Formate Lyase (glycyl radical dependent enzymes); Prostaglandin Synthase (NSAI target). Xylulose Isomerase (Petsko); Triose Phosphate Isomerase (Knowles, Rose). I would be happy to have those of you interested in metallo-proteins chose one of these enzymes. In general the methods to identify intermediates in metallo-protein dependent reactions are more spectroscopically oriented and physical in nature. You may choose your own target protein with my approval.

**On reserve**: Alan Fersht "Structure and Mechanism in Protein Structure" A guide to Enzyme catalysis and protein folding (1999); Richard B. Silverman "The Organic Chemistry of Enzyme Catalyzed Reactions" (1999) and a very old book which describes some concepts very well Christopher Walsh "Enzymatic Reaction Mechanisms"

**All of the readings in bold are on reserve and are required reading**. Additional readings will be suggested at the beginning of each topic and are not required. They also will serve as excellent background reading should you ever need to explore a particular class of enzyme in depth.

There will be four graded problem sets that can be done collaboratively. There will be two exams, closed book, that will be given at night (2 hours for a 1.5 hour exam). These exams will be analogous to the problem sets. There will be a final presentation that will serve as the final exam that will be carried out in the week of class and final exam week.

Grade determination:
1. problem sets (5): 50% (can be done in groups)
2. two exams: 25% (take home exams, open notes, modeled after the problem sets.
3. final presentation: 25% An enzyme not discussed in class whose structure is known will be chosen. You will look at the structure and reaction and use the chemistry you have learned over the course of the semester to propose several mechanistic hypotheses. You will then use the tools you have learned to propose experiments to distinguish between your mechanistic hypotheses. The exam will be one hour during which you will present to me your structure, mechanistic models, and methods to study the system and during which I will ask questions. The exam will be scheduled at your convenience in the last week of classes or during the exam period.

Problem sets will be due September 12 (PS 1); September 24 (PS 2); October 22 (PS 3) and November 5 (PS 4) and December 5 (PS 5)
The two exams will be given at night on October 3 and November 19.

Because I am teaching this course by myself and it was initially scheduled to be taught M, W, F, there will be several classes that I will need to make at a time that will be mutually agreeable to all in the class.