Syllabus for Chemistry 5.50 Fall 2003 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. 4, 9, 11 **Rate Acceleration and substrate specificity**: use of binding energy in catalysis, use of acid/base and nucleophilic catalysis; the stabilization of the transition state vs destabilization of the ground state controversy; chorismate mutases, serine proteases, OMP decarboxylases will be examined as examples to understand catalysis quantitatively.

Assignment: **Fersht Chapter 2, 54-84** and Chapt 12; **Radzicka, Wolfenden Science 267, 90-93 (1995**) "A Proficient Enzyme". **Bruice and Benkovic Biochemistry 39, 6267 (2000)**, "Chemical Basis for Enzyme Catalysis".

Sept. 16, 18, 23, 25 **Proteases**: Post-translational modification by proteolysis plays a major role in apoptosis, blood clotting, hormone production etc. 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.

Assignment: Fersht ,Chapter 3; **Cassidy et al. (1997) Biochemistry 36, 4576-84** "A new concept for the mechanism of action of chymotrypsin: the role of the low barrier H-bond." **Hedstrom et al**. **Science 255, 1249-1253 (1992),** " Converting Trypsin to Chymotrypsin". **Thornberry and Lazebnik Science 281, 1312-1316 (1998), "Caspases: Enemies Within.**

30, Oct. 2 **Phosphoryl Transfer Reactions**: Post-translational modification by phosphorylation plays a major role in signaling in biology. Kinases and phosphatases are the enzymes that regulate the state of protein phosphorylation. Kinases also play a major role in maintaining ATP (energy) levels within the cell. 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.

Assignment: **Herschlag, D**. "Mapping the transition state of ATP hydrolysis: implications for enzymatic catalysis" **Chem Biol 1995 11,729-39;**

Lahiri, Zhang, Dunnaway-Mariano and Allen "The pentacovalent phosphorous intermediate in phophoryl transfer" **Science 2003, 299, 2002-2003. Choe, Ianou, Fromm, Hanzatko,** "Metaphosphate in the active site of Fructose 1,6-bisphosphatase" **J. Biol. Chem. 278, 16015-16020 (2003).**

Oct. 7, 9, 16, 21 **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 to understand ts structure and positional isotope exchange as evidence for intermediates 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.

> Assignment: **Heline et al, Science 294, 369-374 (2001)** "Observation of Covalent Intermediates in and Enzyme Mechanism at Atomic resolution." **Walsh, Enzymatic Reaction Mechanisms p. 711-715 describes chiral methyl analysis; Long, Casey, Beese Nature 2002, 419 645-650 (2002**) "Reaction path of protein Farnesyl Transferase at atomic resolution". **Tobin, Pickett, Fierke, Penner-Hahn, JACS 125, 9962-9969 (2003),** "Structural Characterization of the Zinc Site in Protein Farnesyl Transferase".

Oct. 22, 27 **Isomerases**: The general strategy for enzyme-catalyzed abstraction of α-protons of carboxylic acids (extremely non-acidic hydrogens) will be discussed. Mandelate Racemase (MR) and enolase will be used as prototypes. An introduction to the use of genomics and an understanding of chemistry to identify new function from unknown sequences will be presented using enolase and MR. The concept of superfamilies and approaches to identification of new superfamilies will be presented.

Assignment: **Babbitt and Gerlt**, "Divergent evolution of enzymatic function: mechanistically diverse superfamilies and functionally distinct suprafamilies**", Annu. Rev. Biochem. 70, 209-246 (2001).**

Oct. 30, Nov. 4 **Pyridoxal Phosphate Requiring Enzymes**: General principles of this cofactor chemistry and the structural diversity of these enzymes. D and L Aspartate Aminotransferase will be used 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. 6, 13, 18, 20 **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) and Sir2 (NAD), p-Hydroxybenzoate Hydroxylase (FAD) and Plasma amine oxidase (TOPA quino cofactor) 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.

Assignment: **Massey (1994) J. Biol Chem. 269, 22459-22462**. **Wilmot, C.M, Pearson, A R. Current Opinion in Chemical Biology 6, 202-207 (2002),** "Cryocrystallography of Metallo protein Reaction Intermediates; **Mure, M., Mills, SA, Klinman, J. Biochemistry 2002 41, 9269-78,** "Catalytic Mechanism of TOPA quinone Copper

Containing Amine Oxidases; **Klinman (1989) TIBS 14, 368**, "Tunneling in enzymatic reactions".

Nov. 25, Dec. 2, 4 **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.

Assignment**: Frey, P. A. Ann. Rev. Biochemistry 2001, 70, 121-148**, "Radical mechanisms in enzyme catalysis."

Dec. 9 **Adenosylcobalamin and Methylcobalamin dependent Chemistry**: Homolytic and heterolytic 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.

Assignments: **Toraya, CMLS, Cell Mol Life Science. 57, 106-27 (2000).** "Radical catalysis of B12 enzymes: structure, mechanism, inactivation and reactivation of diol and glycerol dehydratase."

Choices of enzymes for final examine presentation: Uracil Glycosylases (Tainer); Histidine Ammonia Lyase (Retey); Glycosidases (Whithers); Amidotransferases (Smith, J. and Holden, H.); Terpene cylases (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 old 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 five problem sets that can be done collaboratively. Answers to these problem sets will be given out and discussed in a recitation section that will be optional. There will be three exams, closed book, that will be given at night (2 hours for a 1.5 h exams). These exams will be analogous to the problem sets. They will be held on **Sept 25, Oct 27 and Dec 4**. There will be a final presentation that will serve as the final exam that will be carried out in the last week of class or during final exam week as you choose.

Grade determination:

1. three exams: 75%(modeled after the problem sets).

2. final presentation: 25% An enzyme not discussed in class whose structure in the presence and absence of interesting ligands 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 about the chemical mechanism and the mechanisms of rate acceleration. You will then use the tools you have learned in class to propose experiments to distinguish between your mechanistic hypotheses. The exam will be several hours 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 three exams will be given at night on **Sept 25, Oct 27 and Dec4**.

Because I am teaching this course by myself there may be several classes that I will need to make at a time that will be mutually agreeable to all members in the class.