BCHM 101a
Advanced Biochemistry: Enzyme Mechanisms
Fall 2018
Oprian

Four-Credit Course (with three hours of class-time per week)
Success in this 4 credit hour course is based on the expectation that students will spend a minimum of 9 hours of study time per week in preparation for class (readings, papers, discussion sections, preparation for exams, etc.).

Course Information

Meets: MWTh, 10:00 -10:50 am

Recitation: M, 5:00 – 5:50 pm

Instructor: Dan Oprian, office Volen 407, oprian@brandeis.edu

Office Hours: M 1-3

Focus of the Course: Chemistry of Enzyme Active Sites, taught from the point of view of understanding the experiments needed to elucidate mechanism.

Texts: This is an inherently advanced topic course and will be taught exclusively from primary and secondary literature (papers will be posted on the LATTE site). I put five texts on reserve to provide a source of background and reference material:

> Silverman, The Organic Chemistry of Enzyme-Catalyzed Reactions
> Fersht, Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding
> Jencks, Catalysis in Chemistry and Enzymology
> Segel, Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems
> Moore and Pearson, Kinetics and Mechanism

Grades: Grades will be determined on the basis of 4 exams (3 midterms and 1 final, each worth 100 pts; the final will be cumulative), 3 quizzes (worth a total of 100 pts), and 4 problem sets (worth a total of 100 pts). The problem sets will not be graded, but answer keys will be posted, and I will be happy to go over any questions you may have.

Syllabus

Aug 29 (W)	**Overview of Class: Enzymatic Rate Enhancements.**
30 (Th)	**Review of Carbonyl Chemistry.** Latte: Overview of Carbonyl Chemistry
Sept 3 (M)	No class
5 (W)	**Chemical Kinetics.** Review: ln and e Empirical rate laws: From where do they come? 1st-order, 2nd-order, and reversible reactions. Background sources: Jencks, Chapter 11; Moore and Pearson, Chapters 2 & 8. Latte: Integrating factors
6 (Th)	**Chemical Kinetics, cont.** Quiz 1
10 (M)	No class
12 (W)	**Transition-State Theory.** Arrhenius, Eyring, and the Hammond Postulate. The transition state in equilibrium with ground state.
13 (Th)	**General Mechanisms of Catalysis.** Intramolecular catalysis ("approximation"), covalent catalysis (nucleophilic, electrophilic), General Acid-Base Catalysis (GABC), electrostatic, strain, etc. Background sources: Fersht, Chapter 2; Jencks, Chapters 1, 2, 3, & 5.
17 (M)	**Aspartate Aminotransferase – GABC** Linear free energy relationships – Bronsted plots. Assigned reading: Toney & Kirsch (1989) Science 243, 1485-1488. Problem set 1 due Tuesday Sept 18
19 (W)	No class
20 (Th)	**Enzyme Kinetics: Steady-State.** Latte: Review Basic Enzyme Kinetics Background sources: Fersht, Chapter 3.
24 (M)	No class

	25 (T)	**Enzyme Kinetics: Steady-State.** Partition Analysis: Linear and Branched Pathways Assigned reading: Cleland (1975) Biochemistry 14, 3220-3224.
	26 (W)	**Chymotrypsin - Covalent Catalysis.** Burst kinetics (Hartley & Kilbey), common intermediates (Bender), and peptides (Fersht). Assigned reading: Fastrez & Fersht (1973) Biochemistry 12, 2025-2034.
	27 (Th)	**EXAM I**
Oct	1 (M)	No class
	3 (W)	**Chymotrypsin – Covalent Catalysis.** Catalytic Triad (Koshland, Blow, Wells, Abeles, & Petsko) Assigned reading: Carter & Wells (1988) Nature 332, 564-568; Rasmussen, Stock, Ringe, & Petsko (1992) Nature 357, 423-424.
	4 (Th)	**Acetoacetate Decarboxylase - The Enzyme that Created Israel.** Active-site Lys, and perturbed pKa. Background sources: : Schmidt & Westheimer (1971) Biochemistry 10, 1249-1253.
	8 (M)	**Acetoacetate Decarboxylase - The Enzyme that Created Israel.** Structure of the enzyme. Assigned reading: Ho, Menetret, Tsuruta, & Allen (2009) Nature 459, 393-397.
	10 (W)	**Transition-State Stabilization.** And transition-state analogs as inhibitors. Assigned reading: Wolfenden (1972) Accts. Chem. Res. 5, 10-18. Further reading: Schramm (1998) Annu. Rev. Biochem. 67, 693-720; Schramm (2011) Annu. Rev. Biochem. 80, 703-32.
	11 (Th)	**Lysozyme: Covalent Catalysis or Oxocarbenium Ion Intermediate.** Assigned reading: Kirby (2001) Nat. Struct. Biol. 8, 737-739; Vocadlo, Davies, Laine, & Withers (2001) Nature 412, 835-838. Quiz 2

	15 (M)	**Lysozyme: Covalent Catalysis or Oxocarbenium Ion Intermediate, cont.** Assigned reading: Gandour (1981) Bioorganic Chemistry 10, 169-176.
	17 (W)	**Kinetic Isotope Effects (KIE).** Harmonic oscillator, zero-point energy, and primary kinetic isotope effects. Background sources: Westheimer (1961) Chemical Reviews 61, 265; Northrop (1975) Biochemistry 14, 2644-2651.
	18 (Th)	**Kinetic Isotope Effects (KIE).** Secondary isotope effects, inverse isotope effects, and tunneling.
	22 (M)	**Racemases.** One base or two? Assigned reading: Cardinale & Abeles (1968) Biochemistry 7, 3970-3978
	24 (W)	**Racemases.** Enzyme inhibitors – transition-state analogs. Assigned reading: Buschiazzo et al. (2006) PNAS 103, 1705-1710. Background sources: Kenyon & Gerlt (1991) Biochemistry 30, 9255-9263.
	25 (Th)	**Triose Phosphate Isomerase (TIM) – The Perfect Enzyme.** Assigned reading: Knowles & Albery (1977) Acc. Chem. Res. 10, 105-111; Knowles (1991) Nature 350, 121-124. Problem set 2 due
	29 (M)	**EXAM II**
	31 (W)	**Triose Phosphate Isomerase (TIM) – The Perfect Enzyme, cont.** Background sources: Richard (2012) Biochemistry 51, 2652-2661.
Nov	1 (Th)	No class
	5 (M)	**Pyridoxal Phosphate.** Mechanism-based inactivators ("suicide substrates").

Assigned reading: Faraci & Walsh (1989) Biochemistry 28, 431-437.
Background source: Fersht, Chapter 9.
PLP-dependent enzymes.

7 (W) **Pyridoxal Phosphate.**
Assigned reading: Eliot & Kirsch (2004) Annu. Rev. Biochem. 73, 383-415.

8 (Th) **Phosphoryl Transfer Reactions: Why Nature Choose Phosphate.**
Assigned reading: Westheimer (1987) Science 235, 1173-1178.

12 (M) **Phosphoryl Transfer Reactions: Associative, Dissociative, and Concerted.**
Assigned reading: Knowles (1980) Annu. Rev. Biochem. 49, 877-919;
Background source: Herschlag (2011) Annu. Rev. Biochem. 80, 669-702.

14 (W) **Phosphoryl Transfer Reactions: Detection of Intermediates.**
βγ bridge/β non-bridge scrambling and chiral phosphate.
Background sources: Midelfort & Rose (1976) J. Biol. Chem. 251, 5881-5887; Jones et al. (1978) Nature 275, 564-5. Rose et al. (1974) J. Biol. Chem. 249, 5163-5168.

15 (Th) **Thiamine Diphosphate.**
Assigned reading: Kern et al. (1987) Science 275, 67-70.
Quiz 3

19 (M) **Thiamine Diphosphate.**
Assigned reading: Tittmann et al. (2003) Biochemistry 42, 7885-7891; Tittmann et al. (2006) Nat. Chem. Biol. 2, 324-328.

21 (W) No class

22 (Th) No class

26 (M) **Economics of Enzyme Catalysis: Binding Energy.**
Circe Effect
Background sources: Jencks (1975) Adv. Enzymol. And Related Areas of Molecular Biology 43, 219-410; Jencks (1987) CSHSQB 52, 65-73; Stoddard & Baker (2009) Nature 461, 1300-1304.

28 (W) **Free Energy Profiles.**
Background sources: Fierke et al. (1987) CSHSQB 52, 631-638.Anderson & Johnson (1990) Chem. Rev. 90, 1131-1149; Toney (2013) Biochemistry 52, 5952-5965.

29 (Th) **Oxidation-Reduction Reactions: P-450: Nature's Blowtorch.**
A radical mechanism.
Assigned reading: Sligar & Petsko (2000) Science 287, 1615-1622; Sligar (2010) Science 330, 924-925.
Kinetic crystallography.
Background sources: Bourgeois & Royant (2005) Curr. Opin. Struct. Biol. 15, 538-647; Frey (2006) Nat. Chem. Biol. 2, 294-295; Wille et al., (2006) Nat. Chem. Biol. 2, 324-328; Johnson (2012) Nature 487, 177-178; Nakamura et al., (2012) Nature 487, 196-201.
Problem set 3 due

Dec 3 (M) **Oxidation-Reduction Reactions: P-450: Nature's Blowtorch.**
Capturing the intermediate.
Background sources: Rittle & Green (2010) Science 330, 933-937; Krest et al., 2013) J. Biol. Chem. 288, 17074-17081.

5 (W) **EXAM III**

6 (Th) **Oligosaccharyl Transferase: Asn-X-Ser/Thr.**
What nucleophile?
Assigned reading: Locher (2011) Nature 474, 350-356.

10 (M) **Terpene Synthases: Generation and Control of Carbenium Ions.**
Background sources: Davis and Croteau (2000) Topics in Current Chemistry 209, 53-95; Christianson (2017) Chem. Rev. 117, 11570-11648.
Problem set 4 due