Chemistry 371 – Biochemistry Laboratory

Course Description:

During the past century scientists have developed the technology to study biology with increasingly fine resolution. Biochemistry is the study of biology at molecular resolution. In this advanced laboratory course, students will learn to use modern biochemical techniques to isolate, purify, and characterize the enzyme alkaline phosphatase from *E. coli.* To gain insight into the catalytic mechanism of this enzyme, we will perform extensive kinetic analysis, study its 3-dementional structure and consider primary literature reports regarding the kinetic behavior of the enzyme with engineered mutations. The product of your laboratory work for the semester will be a detailed laboratory report and an interactive JMOL tutorial.

Instructor and Resources:

Instructor:	Dr. John Hofferberth
Email:	hofferberthj@kenyon.edu
Office:	312 Tomsich Hall
Office Hours:	TBA
Room:	Tomsich 206
Class Time:	Thursday, 8:10 – 11:00 AM
Text:	Fundamental Laboratory Approaches for Biochemistry and Biotechnology 2nd Ed; Ninfa, Ballou, and Benore.
Website:	moodle.kenyon.edu (search for Chemistry 371 once you have logged in)

Course Policies and Expectations:

Course Grade: Your grade in the course will be determined by your performance on the items tabulated below:

Weekly Reports (10 × 20 pts)	200 pts
Final Report	100 pts
JMOL Tutorial	50 pts
Lab Technique and Participation	20 pts
Total Points	**370 pts**

Attendance: *Attendance at all laboratory sessions is mandatory.* If you know that you will miss more than one laboratory session due to scheduled event please contact me immediately.

A number of the experiments will require lab work outside of normal class hours. It is expected that you do the work needed to complete all of the experiments. The instructor will be available for guidance when additional time is required.

Preparation: Accompanying the description of each experiment in the laboratory manual is a reading assignment from the course textbook. This reading assignment and

the laboratory manual provide sufficient background for each experiment. Students are expected to arrive in the lab *ready to perform the laboratory work*. Students should have prepared their laboratory notebook prior to each experiment (see below) to allow the lab work to be efficiently completed. Each week there will be a very brief pre-laboratory discussion to inform students about safety, use of new instrumentation and equipment, demonstrate new techniques and detail any changes to the experimental procedure in the lab manual.

Laboratory Notebook: Students will maintain a research-style laboratory notebook. A defining characteristic of a good laboratory notebook is that someone trained in the field could repeat the experimental work using only the notebook. The purpose, results, and conclusion of each experiment should also be clear. There will be no specified format for your laboratory notebook, however the course textbook provides excellent guidance (pg 9). It is expected that students prepare their laboratory notebook before arriving for lab.

Weekly Reports: Following each experiment in the laboratory manual is description of the materials that each laboratory team must submit at the beginning of the next laboratory meeting. In all cases, these weekly reports include carbon copies of the laboratory notebook pages in addition to other items pertinent to the experiment of concern.

Final Report: Each team will prepare a journal-style laboratory report describing their work on Alkaline Phosphatase.

JMOL Tutorial: The structure of Alkaline Phosphatase is available and students will use this structure, their own kinetic data, and published reports of the kinetics of engineered mutants to explore the catalytic mechanism of the enzyme. This endeavor will be presented in the form of an interactive JMOL tutorial.

Accommodations: In accord with ***Section 504 of the Rehabilitation Act of 1973 and the Americans with Disabilities Act of 1990***, if you have a disability and need accommodation in order to fully participate in this class, please identify yourself to Erin Salva, Coordinator of Disability Services (PBX 5145, salvae@kenyon.edu). All information and documentation of disability is confidential. The instructor must receive information regarding the nature of the accommodation directly from Erin Salva.

Academic Integrity: Academic integrity is expected in all aspects of this course. A detailed description of academic integrity and the College policy regarding academic dishonesty can be found at the following link: http://www.kenyon.edu/x11747.xml

Schedule of Experiments:

1/21 - Check-in and Purification of Alkaline Phosphatase: cell lysis, clarification/removal of insoluble fraction, dialysis

1/28 - Purification of Alkaline Phosphatase: heat denaturation of impurities and concentration of sample by centrifugation

2/4 - Purification of Alkaline Phosphatase: anion-exchange chromatography using a DEAE column

2/11 - Determination of protein concentration by BCA and Bradford Method

2/18 - Identification of purified Alkaline Phosphatase by gel electrophoresis: SDS denaturing gel

2/25 - Identification of purified Alkaline Phosphatase by gel electrophoresis: native electrophoresis with activity based stain

3/4 - Kinetic Analysis of Alkaline Phosphatase: Determination of kinetic parameters at pH 8 (K_m, V_{max}, and k_{cat}) and determination of optimal temperature

3/25 - Kinetic Analysis of Alkaline Phosphatase: Analysis of the impact of pH on kinetic parameters (each group will test at pH 6 and 10)

4/1 - Kinetic Analysis of Alkaline Phosphatase: Characterization of inhibition by inorganic phosphate, *p*-nitrophenol, and L-phenylalanine.

4/8 - Kinetic Analysis of Alkaline Phosphatase: Characterization of the impact of cofactor concentration on the relative activity of AP – Mg^{+2}, Zn^{+2}, Mn^{+2}

4/15 - Kinetic Analysis of Alkaline Phosphatase: Bioinformatics approach to understanding the catalytic mechanism of Alkaline Phosphatase

4/22- Chapter 12 -Ligand Binding Lab (Pg 275-305) – only Experiment 11-1

4/29 - Chapter 12 -Ligand Binding Lab (continued) (Pg 275-305) – only Experiment 11-1

5/6 - Writing workshop – Peer review of AP lab report; Checkout