Chemistry 371: Advanced 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.* We will perform extensive kinetic analysis, study its 3-dimentional 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, manuscript-like, laboratory report and a guided project focused on the active site of alkaline phosphatase.

What to expect

From the course – Unlike other laboratory courses, each experiment builds upon or supports the work completed in subsequent weeks. This is how "real" research is typically performed. You will be reading primary literature to help you form hypotheses and validate your results. You will also be completing a project outside of class that will help you understand data collected later in the semester. Ultimately, your goal is to compile data and draw conclusions in order to prepare a full laboratory report, similar to a manuscript being prepared for publication. Throughout the semester you will submit portions of this report as a way to prepare the final report and fine tune your scientific writing skills.

From the instructor – to treat each person with respect, arrive to class on time and prepared, return graded and assessed items in a timely manner, reply to emails within 36 hours (however, I will not reply after 9 pm), and be available outside of class for questions or further discussion.

Of the students – to respect others, be on time (when arriving to class, turning in assignments, etc.), and be prepared for class (read the background material, notebook up-to-date, and ready to perform the experiment).

Instructor and Course Resources

Instructor:	Prof. Kerry Rouhier ("Roo-yer")
Email:	rouhierk@kenyon.edu
Office:	212 Tomisch Hall
Office Hours:	Monday (10 AM – 12 PM), Wednesday (10 AM – 11 AM), Thursday (8 AM – 10 AM)
Class location:	Pre-lab – Tomsich 206; Lab – Tomsich 203
Class time:	1:10-4:00 pm Wednesday
Text:	Laboratory notebook with carbon pages and *Fundamental Laboratory Approaches for Biochemistry and Biotechnology* 2nd Ed; Ninfa, Ballou, and Benore.
Websites:	*moodle.kenyon.edu* and *Turnitin.com*

Course Policies

Grade – grades will be determined using the following absolute scale and distribution of points (A/A-: 90-100%; B(+/-): 80-89%; C(+/-): 70-79%; D(+/-): 60-69%; F: <60%). The instructor reserves the right to alter this grading scale.

Notebook checks (9 @ 6 pts)	54 pts
Weekly Quizzes (6 @ 5 pts)	30 pts
Post-lab assignments (8 @ 20pts)	160 pts
Introduction and Bibliography Draft	50 pts
Active Site Project	50 pts
Journal-style Manuscript	150 pts
Peer Editing Workshop	30 pts
Total	**524 pts**

Attendance – *Attendance at all laboratory sessions is mandatory.* If you know that you will miss a laboratory session for a scheduled event please contact the instructor immediately. I reserve the right to expel students from this course for excessive absences. 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.

Accommodations – In accord with Section 504 of the Rehabilitation Act (1973) & ADA (1990): If your disability requires accommodation to fully participate in this class, notify the instructor and Erin Salva, Coordinator of Disability Services (PBX 5453, salvae@kenyon.edu) as soon as possible. All information and documentation of disability is confidential. No accommodations of any kind will be given in this course without notification from the Coordinator of Disability Service at least one week prior to the special accommodations.

Academic Honesty: Academic honesty is expected in all aspects of this course. Please refer to the Course of Study 2013-2014 (linked on the Moodle site) for a detailed description of the policies on academic honesty. A violation of academic honesty is among the most serious matters in an academic community. Note: this class encourages collaborative work; however your work must still be your own.

Late work – Late work will be accepted with a penalty of 10% per 24 hours late (including weekends). Work more than 5 days late will not be accepted.

Technology use – Laptops will be provided (or you may use your own) at each class to work up data. Please refrain from using it to email, instant message, play games, or any other activity unrelated to class. Cell phones are not to be used during class. Please have them turned off or set to silent.

Course Details

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 should have prepared their laboratory notebook prior to each experiment (see below) to allow the lab work to be efficiently completed.

Quizzes – On the dates indicated in the syllabus, there will be an in-class quiz based on background reading sections and experimental details for the lab that will be accomplished on that day. The quiz will take place from 1:10 to 1:15 PM (no make-up or extensions will be given). Quizzes will be closed-note.

Laboratory notebooks – 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. Rigorous documentation of activities in the laboratory is an essential component of experimental science. A detailed description of the expectations for your laboratory notebook can be found in Ninfa, Ballou and Benore (pgs 9-12). You need to read this section of the textbook carefully before arriving to the laboratory for Week 2 experiments. You will be working **with a partner** for the experimental work in this course and it is expected that you will divide responsibilities. However, every notebook should contain sufficient information to repeat the experiment. All notebooks should contain all of the sections described in the textbook (Purpose, Brief Overview, Materials, Procedure, Results, Discussion/Conclusion). Notebooks will be submitted and checked nine times throughout the semester. Unless noted differently, **notebooks are due on Friday following Wednesday's lab by 12 PM**. Your notebook will be ready for pick up by 4 PM that day. See the schedule for specific due dates.

Post-Lab assignments – At the completion of each experiment, **each pair** of students will prepare a post-lab report. The specific contents of the report will vary from experiment to experiment but will generally include a journal-style Materials and Methods section and Results section. A sample publication (Biochemistry **2009,** *49*(1), 207-217) is linked to the Moodle site and is to be used as a style guide. The purpose of the post-lab assignments is for you to practice writing the *Materials and Methods* and *Results* sections that will appear in your final cumulative report. Unless noted, **post-lab assignments will be due the following Monday by 12 PM** through the Moodle site**.** One person from each pair is to upload their post-lab to the Moodle site. See the schedule for specific due dates.

Introduction and Bibliography draft – During the first several weeks of the course you will use resources from the course and primary literature articles to inform yourself about the enzyme we are studying. Using this information, **each student** will write an introduction section for the final cumulative report. The purpose of this assignment is to give you practice writing the *Introduction* and *References* section that will appear in your final cumulative report. **This draft is due October 9, 2013 by 12 PM** through the Moodle site.

Active Site project – **Each pair** of students will investigate the protein sequence, active site, and catalytic mechanism of alkaline phosphatase. The results of this guided investigation will be submitted through the Moodle site. Knowledge gained from this project will need to be included in your final report. **This project is due September 27th by 12 PM** to the Moodle site.

Final Report – **Each student** will prepare a manuscript that mimics the style of an article published in *Biochemistry*. The course textbook gives a nice description of the components of a 'Laboratory Report' which will guide you in preparation of the report (pgs 13-14). A good way to learn a discipline specific writing style is to read several articles in the current literature. This can be accomplished by visiting the webpage for the journal *Biochemistry* (http://pubs.acs.org/journal/bichaw). **This is due Wednesday, December 18, 2013 by 4 PM.**

Schedule

Date (group pairing)	Experiment	Quiz	Notebook checks	Post-lab due dates
9/4 (I)	1: Intro, safety, check-in, make solutions		9/6 (Friday, 12 pm)	
9/11 (I)	2: Isolate AP	Quiz 1	9/13 (Friday, 12 pm)	9/16 (Monday, 12 pm)
9/18 (I)	3: Purify AP part 1	Quiz 2	9/20 (Friday, 12 pm)	9/23 (Monday, 12 pm)
9/25 (I)	4: Purify AP part 2	Quiz 3	9/27 (Friday, 12 pm)	9/30 (Monday, 12 pm)
10/2 (I)	5: Determine [protein]	Quiz 4	10/4 (Friday, 12 pm)	10/ 7 (Monday, 12 pm)
10/9 (I)	6: SDS-PAGE and native page	Quiz 5	**10/14 (Monday, 12 pm)**	10/14 (Monday, 12 pm)
10/16 (II)	7: Kinetic analysis with PNPP	Quiz 6	**10/21 (Monday, 10 am)**	**10/22 (Tuesday, 4 pm)**
10/23 (II)	8: Kinetic analysis with inhibitor			
10/30 (II)	9: Kinetic analysis with inhibitor		**10/30 (Wednesday, 1 pm)**	11/4 (Monday, 12 pm)
11/6 (III)	10: Kinetic challenge			
11/13 (III)	11: Kinetic challenge		11/15 (Friday, 12 pm)	
11/20 (III)	12: Kinetic challenge			
11/27	Thanksgiving break			
12/4 (III)	13: Kinetic challenge			**12/6 (Friday, 12 pm)**
12/11	14: Wrap up experiments, peer editing, and checkout			
12/16-21	Final exam week – final report due on **Wednesday (18th) at 4 pm**			

You must be flexible in lab to accommodate errors, unexpected delays, and adjustments to protocols. We will adjust the schedule as needed. In some cases you may need to repeat a procedure to obtain acceptable results. If you plan ahead each week for your lab work, you will be able to complete all the course objectives in the time provided.