

## Chemistry 371: Advanced Biochemistry Laboratory

### Course description

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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-dimensional 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

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From the course – Unlike many 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 manuscript as if it were being submitted for publication. Throughout the semester you will submit portions of this manuscript as a way to prepare a polished manuscript 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

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Instructor:	Prof. Kerry Rouhier (“Roo-yer”)
Email:	rouhierk@kenyon.edu
Office:	212 Tomisch Hall
Office Hours:	Monday (10 AM – 12 PM), Tuesday (12:30 PM – 1:30 PM), Thursday (3 PM – 4 PM), Friday (10 AM – 11 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 <i>Fundamental Laboratory Approaches for Biochemistry and Biotechnology</i> 2 <sup>nd</sup> Ed; Ninfa, Ballou, and Benore.
Websites:	<a href="http://moodle.kenyon.edu">moodle.kenyon.edu</a> and <a href="http://Turnitin.com">Turnitin.com</a>

## Course Policies

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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 (8 @ 5 pts)	40 pts
Weekly Quizzes (8 @ 6 pts)	48 pts
Manuscript section drafts (3@ 40 pts, 1 @ 50pts)	170 pts
Active Site Project	50 pts
Submitted Manuscript	150 pts
Peer Editing Workshop	50 pts
<b>Total</b>	<b>508 pts</b>

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 – Students who anticipate a need for accommodations in this course because of the impact of a learning, physical, or psychological disability are encouraged to meet with me privately early in the semester to discuss their concerns. In addition, **students must contact Erin Salva, Director of Student Accessibility and Support Services** ([740-427-5453](tel:740-427-5453) or [salvae@kenyon.edu](mailto:salvae@kenyon.edu)), as soon as possible, to verify their eligibility for reasonable academic accommodations. Early contact will help to avoid unnecessary inconvenience and delays. 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: Kenyon College is, at the core, an intellectual community of scholars – students and faculty – engaged in the free and open exchange of ideas. Critical to this lively exchange and deep engagement with ideas is the academic integrity of our work, both inside and outside the classroom. At Kenyon we expect all students, at all times, to submit work that represents these standards of academic integrity. It is the responsibility of each student to learn and practice the proper ways of documenting and acknowledging those ideas and words you have drawn upon (*see Academic Honesty and Questions of Plagiarism in the Course Catalog*). Ignorance and carelessness are not excuses for academic dishonesty. For this class, students have permission to use or copy material from the manual as needed, but all manuscript drafts and submitted projects must be your own work. If you are uncertain about the expectations for academic honesty in this class, please ask for clarification.

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 for any reason. Please have them turned off or set to silent. Please bring a calculator to each class.

## Course Details

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Lab Manual – The manual is located as a linked file on the class Moodle site. A print copy of the manual will be made available as a reference during laboratory periods.

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 during pre-lab. The quiz will take place from 1:10 to 1:15 PM (no make-up or extensions will be given). Each quiz will ask the same three questions: (1) What is today's experimental goal? (2) How will you accomplish that goal? (3) How will you know you were successful?

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 eight times throughout the semester to ensure data is worked up correctly and appropriate conclusions are made (see sample assessment rubric). See the schedule for specific due dates and times.

Active Site Project – **A 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 support data collected during the kinetic experiments and can be used in the final manuscript. **This project is due September 24<sup>th</sup> by 8 PM** to the Moodle site.

Manuscript sections – One of the major goals of this course is to learn how to assemble and write a manuscript intended for publication in the journal *Biochemistry*. Most scientists prepare their manuscripts in sections – Introduction, Methods, Results, Discussion, References, but not necessarily in that order. You will be writing a manuscript based on the experimental work you conduct in the lab and results you obtain. **Each section draft of the manuscript is due by 8pm the day of lab** through the Moodle site. Even though you are completing the experiments as a team, you will be writing and submitting your manuscript individually. Complete manuscript will be reviewed by a peer editor on **December 10 during class**. Submissions (by 1pm, December 10) and reviews (by 4pm, December 10) will be completed through the class Moodle site.

Submitted manuscript – **Each student** will prepare a manuscript that could be submitted to the journal *Biochemistry*. The course textbook gives a nice description of the components of a ‘Laboratory Report’ which will guide you in preparation of the manuscript (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>). **The final, polished manuscript is due Wednesday, December 17, 2014 by 4 PM.**

### Schedule

Date	Group	Experiment	Quiz	Assignments (due 8pm day of lab)	Notebook Checks	
9/3	1	1: Intro, safety, check-in, make solutions				
9/10	1	2: Isolate AP	1		9/12 <b>(Friday, 12pm)</b>	
9/17	1	3: Purify AP part 1	2		9/19 <b>(Friday, 12pm)</b>	
9/24	1	4: Purify AP part 2	3	Active Site Project due	9/26 <b>(Friday, 12pm)</b>	
10/1	1	5: Determine [protein]	4	Methods: isolation and purification of AP	10/3 <b>(Friday, 12pm)</b>	
10/8	1	6: SDS-PAGE and native page	5		10/10 <b>(Friday, 12pm)</b>	
10/15	2	7: Kinetic analysis with PNPP	6	Results: purification table, [AP] and SDS-PAGE	10/20 <b>(Monday, 12pm)</b>	
10/22	2	8: Kinetic analysis with inhibitor	7			
10/29	2	9: Kinetic analysis with inhibitor			10/31 <b>(Monday, 12pm)</b>	
11/5	3	10: Kinetic challenge	8	Discussion: kinetic analysis		
11/12	3	11: Kinetic challenge				
11/19	3	12: Kinetic challenge		Introduction and Reference Sections	11/21 <b>(Friday, 12pm)</b>	
11/26		Thanksgiving break				
12/3	3	13: Kinetic challenge and wrap-up				
12/10		14: Peer editing of final manuscript and checkout				
12/17		Polished manuscript due by <b>Wednesday (17<sup>th</sup>) at 4 pm</b>				

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.