

Syllabus

Department of Chemistry
FCH 531 – Graduate Studies
Biochemistry Lab

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BIOCHEMISTRY LAB
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FCH 531

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Welcome to Biochemistry Laboratory. This course is an advanced laboratory in biochemical techniques. Unlike most laboratories, which are geared to the slowest student, this laboratory is geared to how fast a practicing biochemist does experiments. Be prepared and expect to spend considerable time in the lab.

Textbook: Biochemistry Lab Experiments, A Revised Manual
G.L. Boyer, R.J. Speirs. et. al. 2000 edition.

No additional text books are required. Two suggested reading are on reserve in the library:

Biochemical techniques; Theory and Practice.
J.F. Robyt, B.J. White, Waveland Press Inc., 1990.

Successful Lab reports, A Manual for Science Students
C.S. Lobban & M. Schefter, Cambridge University Press., 1992.

Grading: Grading will be based on a combination of written reports and exams. Roughly half the scores will come from written material including lab reports and exams, and half the score will come from a subjective score based on your performance and preparation in the lab. In general, lab reports are not graded on your final results, but on how you present and discuss those results

Notebook; 80 points (20%): In an effort to cut down on the paper-work associated with this laboratory, we will not require laboratory reports for each experiment. Instead, you are required to keep a research grade laboratory notebook. This notebook must be bound and all pages must be consecutively numbered. You should bring it to the first lab. Please leave the first page blank. This page will be used by the TA or laboratory instructor to initial off completed experiments. Page 2 is your Table of Contents. The notebook may be used during all quizzes and examinations.

Formal Lab reports; 120 points (30%): You will prepare formal laboratory write-ups for three experiments;

Experiment 1, Determination of Proteins:	20 points
Experiment 7, Catalase:	40 points
Experiment 14, Isolation of an Enzyme:	60 points

Instructions for the reports will be given during the first part of class. Reports will be prepared in Journal format and must be typed.

Exams; 120 points (25%): We will have the following in class exams to cover theoretical aspects of the course. This is subject to change depending on how I feel the class is doing but roughly is as follows:

Midterm Exam/Quizzes:	40 points
Final Exam (in class):	80 points

Laboratory Skills; 100 points (25%)

Laboratory reports, notebook, exams, and quizzes will form a major portion of your grade. In addition, the TA's and myself will give you a purely subjective grade based on your performance in the laboratory. A large portion of this subjective evaluation will depend on if you are prepared for the lab ahead of time and are doing your fair share of the experiments. A neat up-to-date notebook, handed in on time, will certainly help in this category.

Some useful hints;

- These labs are designed to be relatively short if you have planned ahead. Please pre-read the lab protocol. We spend most of our day in this building and can be very grumpy if we are forced to be late. Grumpy TA's give low grades so keep your TA's happy!
- Food and drink will not be consumed in the laboratory.
- At the beginning of each lab section, there will be a short discussion on the experiment you will be doing that period. Lab will try to start promptly at 1:00. Please get to lab at little early. If you need to get ice for the upcoming experiment – get it before 1:00 ! Any ice obtained after 1:00 must come from the third floor. Be careful with your personal items.
- Food and drink will not be consumed in the laboratory.
- If you are not certain how to use something - Ask! In most cases, we will be using research grade instrumentation (not teaching grade). Automatic pipettes run between \$150-\$350 apiece. They are very easy to break. You will not be charged for normal wear and tear on equipment. You will be charged if I feel any damage was due to carelessness on your part.
- Some reagents are very expensive. Pay attention to how much of a given reagent is used in the experiment so there will be enough for your coworkers. If the experiment calls for 50 ml, take 50 ml. Do not take 200 ml because that is the only beaker you have available.
- A lab coat is optional (but useful). If you see your instructor wearing a lab coat, that should tell you something. In most cases, you will be wearing gloves. Learn to take gloves on and off and when to reuse them. Safety glasses must be worn for certain experiments.
- Accidents make me very upset. We will be using some rather nasty chemicals so BE CAREFUL. If an accident does occur, make certain that the victim (be it people or equipment) is properly cared for and then contact me. I need to know about all accidents, no matter how minor.
- Food and drink will not be consumed in the laboratory.

- In short. This is meant to be a very interactive laboratory. We have a very good student / instructor ratio so feel free to ask us questions at any time. However do not expect us to do your thinking for you. If you plan ahead, things should go very smoothly. If not, it will be a long afternoon for many of you.

What I expect from the students

Grade of “C”

- Attend class
- Contribute to each lab experiment
- Keep and hand in a laboratory notebook
- Hand in all three reports

Grade of “A”

- Contribute equally with your lab partner to each lab experiment
- Have reasonable technical skills and an understanding of the basic concepts behind each experiment as evidenced by your performance in the lab and on exams
- Notebook has both raw data and analysis of that data for each and every experiment. It is presented in a logical fashion that the TA can follow.
- Lab write-ups are grammatically correct and illustration a clear understanding of the different elements of a lab report (introduction, methods, results and discussion)
- You can present and discuss your raw results for the three “formal” write-ups in a manner that convinces me you not only understand the basic principles/goals of the lab, but have actually thought about your results and what they mean, actual sources of error (not the generic dribble that you have put in other lab reports) and what conclusions can be supported by your data.
- All assignments must be handed in on time.

INSTRUCTIONS FOR WRITING OF LABORATORY REPORTS

The hardest thing for a student to do is write a clean and brief laboratory report. Most students think bigger is better. They could not be farther from the truth. The objective is to convey the essential information as briefly as possible. In this course, we will be using "Successful Lab Reports" by C.S. Lobban and M. Schefter as a style manual. Two copies should be on reserve in Moon Library. You should read it prior to writing the first lab report and follow their guidelines.

C.S. Lobban and M. Schefter (1992) Successful Lab Reports; A Manual for Science Students. Cambridge University Press, New York. 106p. [ISBN 0-521-40741-9; \$12.95 as paperback]

For a more advanced treatment for scientific papers, I recommend;

Scientific Writing for Graduate Students (1989) Council of Biological Editors, Inc., Chicago IL 60601. 187p. [\$16.95]

Written reports should be styled as if they are a manuscript submitted for outside publication. They include the following;

Abstract: A 200 word condensed summary of the work.

Introduction: This introduces the problem. Ideally you would present the hypothesis and objectives here. You should review any pertinent background information necessary to understand your results.

Methods: What you did. You may cite the lab manual but must also include a brief summary with any modifications you made in case someone does not have access to the lab manual. Methods should be written so that someone with the same skill level as yourself can repeat the procedure exactly with the information provided. Always write in past tense.

Calculations: This is optional but you may need to explain numerical calculation done in processing the raw data. A sample calculation is useful, especially if you make a mistake.

Results: The "data" section and probably one of the more difficult to write. Proper use of tables and figures will help. Fight the temptation to simply include tables of the "raw" data. Give positive statements about what trends you observed, but do not "discuss" your results.

Discussion / Conclusions: This is the second half the results/discussion section. You must put your results in the framework of current scientific knowledge. Did the experiment work as planned? If not, Why? Could the procedure be improved? Make certain you know the difference between what goes in "Results" and "Discussion". Specific questions raised as part of the laboratory protocol may be more appropriately answered in one or the other. Questions for thought listed at the end of the experiments should not be answered in the report.

You must type the report double-spaced. Please include a title page with abstract (200 word maximum) and start your introduction on page 2. We will have many reports to read in a short period of time and organization, appearance, ease of finding material, and brevity are essential. Avoid the following common problems in your reports:

- Too lazy to read the style manual. This generally shows up as not knowing when to use a figure vs. a table, results in the methods section, and a host of other problems.
- Poor grammar. Don't kid yourself. Poor grammar and misspellings will affect your grade. Use a spell-checker and proof-read your report carefully.
- Incomplete abstract or abstract written in the future tense. The abstract is a summary of what you **did**. It must be in past tense and should include all elements (introduction, methods, results and discussion) of your report. Keep it "to the point".
- Verb tense. The abstract, methods and results are all sections that describe what you did or found and must be written in past tense. Introduction and discussion have more flexibility but should be writing in a consistent tense. The exception is when citing someone else's work where a reported fact is still a fact. In this case, it is proper to refer to it in present tense, i.e. "Mr. Smith reported the Michaelis constant for alcohol dehydrogenase is"
- Capitalize "Table" and "Figure" when used as proper noun in text. Capitalization of these words is often done incorrectly. When you directly refer to a specific figure or table (note lower case), such as Figure 1 or Table 1 (note upper case), you referring to it by its proper name and it must be capitalized.
- Figure legends and Table headings need to "stand by themselves". By this, someone should be able to take the figure out of the report and still understand what it shows purely from the legend. Write the legend of heading in full sentences (subject and verb).
- Using the same data twice. Data can either be given in a figure or a table but not both. Read the style manual and make certain you understand when to use which approach for maximum effect.
- Too much or too little detail in the Methods. You can not simply refer to the experiment as being done as described in experiment 3 since the reader may not have the lab protocol in front of them. At the same time, you do not need to tell the reader how many hairs are on the horse for them to figure out how to ride. Work on a compromise, giving just enough info for the reader familiar with biochemistry to repeat your work.
- Methods in results and results in methods or discussion. Learn what goes where. New data cannot be introduced in the discussion section.
- Conclusions that are not supported by your data. Be certain to discuss your results and draw conclusions that can be supported by your data. You may contrast these to what has been reported in the literature or what is expected, but you do not have the option of saying something was not done correctly because you did get the same results as someone else.
- Assigning blame in the discussion. Write in positive terms (what did your find, not what didn't work), even when discussing errors. You probably don't know what went wrong if it didn't work, so state possibilities, thoughts on why, and let it go.

Biochemistry 531

Name: _____

Question #1: Due first day of lab.

You wish to make up a 1.0 ng/ul standard of the plant hormone abscisic acid (MW = 269 g/mole). You do not have enough of the hormone to weigh accurately so instead, you dissolve what compound you do have in 1.0 ml methanol. Taking a 1 to 10 dilution, you find the UV absorbance is too large to measure. Diluting the sample an additional 5-fold gives an absorbance at 266 nm of 0.45 units (1 cm light path). Knowing the molar extinction coefficient for abscisic acid is 19,900 (266 nm), calculate how much you must dilute the initial 1 ml solution to obtain your 1 ng/ul standard.

BIOCHEMISTRY LABORATORY SCHEDULE
FCH 531: Fall 2009

- Sept 2: Orientation and Check-in, Pick up lab experiments in Room 137 Jahn.
No labs this week (Chemistry day at the fair is Thursday)
- Sept 7: Experiment 1; Determination of proteins
- Sept 9: Experiment 2; Isolation of a peptide (or start Experiment 3)
- Sept 14: Experiment 3; Sequencing a peptide,
- Sept 16: Experiment 3; continued, Discussion of enzyme kinetics
- Sept 21: Experiment 4; Polyphenoloxidase and enzyme kinetics
Write-up for Experiment 1 due Wed Sept 22 (4:30 p.m.).
- Sept 23: Experiment 4; Polyphenoloxidase and enzyme kinetics, continued
- Sept 28: Experiment 5: Hexokinase, coupled enzyme assays
- Sept 30: Experiment 6: Comparison of extraction techniques
- Oct 5: Experiment 7; Isolation of Catalase
- Oct 7: Experiment 7; Isolation of Catalase
Notebooks for Experiments 1-6 (due 4:30 p.m. Wednesday, Oct 6)
- Oct 12: Experiment 8; Polyacrylamide electrophoresis
- Oct 14: Experiment 9; Native gel electrophoresis
- Oct 19: Experiment 10: Protein HPLC (even numbered groups)
Experiment 12: Western Blots (odd numbered groups)
- Oct 21: Experiments 10, 12 (reverse groups)
- October 26rd **Midterm (open notebook)**
- Oct 28: Experiment 14 (through Nov 23): You should be prepared for Individual discussions regarding your protocols starting on the following Tuesday in the lab.
Write-up for Exp. 7&8 due October 30 by 1:00 pm.
- Nov 23: Experiment 14 cont.; last day for lab work

***** Thanksgiving Day Vacation *****

- Dec 2 Lab clean-up and check out (attendance mandatory)
- Dec 7: Discussion and Oral Presentations of Experiment 14
(Rm 122; 1pm) This will go into December 9th if needed
- Dec 13 **Experiment 14 write-up Due Monday December 13th by 4:30 pm**
- Dec ? : **Final will be given in the lab (open notebook) during the assigned lab-exam period. Notebooks are due at end of exam.**