

Chem 112L

Department of Chemistry and Biochemistry

University of California, Santa Barbara

Spring 2013

General course information for Chem 112L, **BIOPHYSICAL AND BIOANALYTICAL LABORATORY**

Lecturer: Kalju Kahn kalju@chem.ucsb.edu Phone: x6157
PSB-N 2623
Office hours: Tue 3:30-4:30 PM, "Open Door" policy other times

Teaching assistants:

MW2: Clayton Woodcock (cwoodcock@chem.ucsb.edu)

TR6: Steve Szabo (iszabo@chem.ucsb.edu)

Lab session times:

Section 1: Monday/Wednesday 2:00-5:50p.m. PSB-N 2619

Section 2: Tuesday/Thursday 6:00-9:50p.m. PSB-N 2619

Course website:

Recommended text: *Same as in Chem112 lecture.*

Course Goals

The purpose of Chem 112L is twofold: (i) introduce you to the instrumentation used in biophysical and bioanalytical chemistry, and (ii) help to understand concepts that are taught in Chem112. Your work is organized around a small number of projects, and most projects will take more than one lab session.

Lectures and discussions that cover topics that are not normally taught in Chem112 will be held on some days.

Course Information and Expectations of Students:

General

Chem 112L is a laboratory course, in which the main portion of your grade is earned by planning and performing experiments, documenting your work in your lab report, and answering questions in the manual. There is a quiz at the beginning of each experiment, two exams throughout the course, and a poster research presentation at the end of the course. As with any laboratory course, standard lab fee is collected from students who stay beyond the standard drop deadline.

Attendance

Attendance in all laboratory sessions is mandatory. Please contact your instructor, your TA, and your lab partner at least one week ahead of time if you have to miss a class. For each missed class you must turn in a signed letter detailing the reason for missing the class. For unexpected misses, you must provide a verifiable documentation stating why you could not make the class. There is a make-up day toward the end of the quarter to repeat missed or unsuccessful experiments. If you are a student with a disability and would like to discuss special academic accommodations, please contact me during my office hours.

Preparation for the lab

Laboratory manuals can be downloaded from the course website. You are expected to be well familiar with material in the course manual as well as with any material presented in the 'required reading' section. You should show an adequate preparation in order to perform the experiment. Your preparation is judged to be inadequate if you receive 35% or fewer points **both** on your quiz and on your prelab for that day. Your preparation is also judged to be inadequate if you receive 25% or fewer points on **either** the prelab or the quiz for that day. If you are not adequately prepared, you are asked to leave the lab. You may make up the experiment during a suitable time if this was the first instance of inadequate preparation. You will receive zero points for the whole experiment on any subsequent instance of inadequate preparation.

Academic honesty

Honesty and academic integrity must be always preserved. It is your responsibility to be familiar with common sense about academic integrity. Some examples of academic dishonesty are:

- a) altering experimental data without clearly explaining the nature of alterations
- b) intentional misrepresentation of the meaning of data
- c) using other student's data as if it was your data
- d) copying information from any source and presenting it as if it was your original answer
- e) copying images from any source and presenting them as if they were created by you
- f) sharing information about quiz questions with other students
- g) altering the experimental set-up of other students without their permission

For additional information about types of disallowed conduct, please see

<http://judicialaffairs.sa.ucsb.edu/PDF/academicintegflyer.pdf>

While working with your partner(s) is encouraged in the laboratory, you must write your notebook up independently. You may discuss prelab questions and additional questions with other students in the class but make sure that your answers are original. Plagiarism will not be tolerated and will result in score zero on that lab report. Cheating in any form will result in a failing grade and notification of Associate Dean of Students, Conduct and Student Relations. No supplemental materials should be used during quizzes and the final exam.

No student shall give, sell, or otherwise distribute to others or publish any electronically available course materials or recordings made during any course presentation without the written consent of the instructor.

Safety

Even though we have had an excellent safety record in our biochemistry laboratory, the teaching laboratory can be a dangerous place. A few hazards that are present this quarter include hot water or hot surfaces, toxic or corrosive chemicals, electricity, operating centrifuges, and broken glass. The general advice to safety is: **know what you are doing by preparing for lab**. Each experiment in the manual outlines most serious hazards that are present while performing the experiment and discusses ways to prevent accidents. Be sure to read these carefully and ask your TA or the instructor if you have any questions. Students have a right to view Material Safety Data Sheets (MSDS) for chemicals used in the class. These can be accessed from <http://ehs.ucsb.edu>. Your TA will remind you of the potential hazards before each class.

You must follow basic safety rules¹ to ensure safety for yourself and fellow students during the class.

- 1) Always wear some sort of protective eyewear. You must wear either lab goggles that protect from the sides as well as from the front or a face shield. You can purchase lab goggles during the first weeks of the class from the bookstore. There are two face shields in the laboratory. If you are wearing normal glasses, wear goggles over them as normal glasses do not provide side-protection. You may wear contact lenses along with lab goggles. Protective eyewear is not required in the computer lab.
- 2) Wear gloves when required, or when you are working with dangerous chemicals. Replace gloves when they become contaminated.
- 3) Wear appropriate clothing in the wet lab. You must wear closed shoes, and a shirt that covers the midsection. Wear long pants, very long skirt, or very long dress on the days that you will be performing experiments. Wear a lab coat when handling chemicals in the laboratory.
- 4) Label all solutions that you prepare clearly. If the solution is in a container larger than 2 mL, make sure that the label conveys information about the content of the container and also identify your group or you personally as the person responsible for this solution. Unlabeled solutions will be discarded.

¹ The course manual is the equivalent to the OSHA Chemical Hygiene Plan. This applies to course TA-s.

- 5) Familiarize yourself with the safety equipment in the lab. Our lab has one fire extinguisher near the back door, and eyewash station near the front door. The first aid kit is in the top right drawer next to the main sink. Emergency phone numbers are posted in the lab. In case of fire, earthquakes, or other major disasters leave the lab if this is safe, and meet your instructor at the lawn south of PSB-N building.
- 6) Do not eat or drink in the laboratory. Closed containers with food may be brought into the laboratory but must be stored in your bag or backpack placed in the cabinet box. You may eat or drink outside the laboratory if time permits.
- 7) Never work alone. Never use mouth suction. Never open the lid of a spinning centrifuge. Do not perform any unauthorized experiments. Come and see your instructor if you would like to perform an additional interesting or fun experiment. He is probably willing to let you do it.
- 8) In case of accident, alert fellow students and immediately take an appropriate action. Explain to your TA what happened and seek further help if necessary.

Ignoring safety rules while in the laboratory will lead to oral warning on the first instance, and deduction of lab quality points for each following instance. Serious intentional violations of safety rules will lead to the dismissal from the course.

Cleanliness

It is important to maintain cleanliness in this laboratory. Even minor impurities on the glassware or on the pipette tip may ruin an otherwise well-done biochemical experiment. For example, using the same pipette tip to transfer two enzymes from their containers into your microcentrifuge tube will most surely contaminate the stock of the second enzyme with the first one and will likely ruin the results for the whole class. You will be working a lot with pipettors that use disposable tips. Discard the tip as soon as you do not need it. They are a lot cheaper than the chemicals that you are working with.

Most used plasticware, such as microcentrifuge tubes or Falcon tubes (15 mL, 50 mL size) are for one-time use. Empty all the tubes before discarding them (it's OK to leave less than 50 μ L in microcentrifuge tubes). Discard broken glassware and used glass pipettes into the red container. Do not discard functional glassware or any parts of the equipment used. If your glassware is visibly dirty, wash it with soap and hot water; otherwise rinse several times with distilled water from the tap, and place on the drying racks. **Do not leave any dishes in the sink.**

Student must label all the containers larger than 5 mL that contain solutions made by them clearly with their name and content of the container. Unlabeled containers will be disposed by your instructor. Each student is responsible for completely cleaning the workplace, washing all the glassware used, and disposing all the disposable plastic-ware.

Your instructor and TA will assign students lab quality points. Each minor violation of lab safety or cleanliness procedures (e.g. inappropriately labeled container, not cleaning the balance area after use, not

wearing gloves when appropriate) leads to a 2-point deduction. Each major violation of lab safety and cleanliness procedures (e.g. leaving dishes in the sink, inappropriate disposal of materials, not cleaning your work area after the lab, not wearing goggles when appropriate) leads to a 5-point deduction. **Leaving a mess will result in a bad grade.**

You will be using some research facilities and equipment during this quarter. Keep them clean as well.

Experiments and lab reports

There are six planned projects in this course:

1. Allantoin conformational analysis: computer modeling and NMR
2. Protein unfolding monitored by circular dichroism (unfolding of lysozyme)
3. Ligand binding by lysozyme: determination of the equilibrium constant
4. Multi-substrate enzyme kinetics and inhibition
5. Electrospray ionization mass spectrometry characterization of proteins
6. Protein crystallography

If a functional stopped-flow instrument is available, pre-steady-state kinetic studies will be demonstrated

Each report must be typed and look professional as if it was a manuscript that you submit for a publication. All the figures should be incorporated into the text and labeled appropriately. Each report should include the following: (approximate percentage of grade in parentheses, see the grading rubric for specific figures):

- title page giving the name of the experiment, your name, and grading rubric (required)
- the objective of the experiment in your own words (3 %)
- the rationale for the experimental set-up (10 %)
- methods: a concise summary of steps taken to accomplish the goal.
Describe sample preparation. (2-3 %)
- answers to the prelab questions (10 %)
- most important data organized as tables, graphs, or images (10-15 %)
- mathematical analysis of data, if applicable (7-8 %)
- a discussion about the physical meaning of your results (20 %)
- a conclusions section summarizing the most important findings (10 %)
- answers to the additional questions given at the end of the experiment, (17 %)
- when appropriate, an appendix with all the raw data that were collected, and all calculations that

you performed. The appendix is not graded but serves as an evidence of your independent work.

The objective of the experiment, the rationale for the experimental set-up, concise summary of the procedure, and answers to your prelab questions constitute the *prelab* that you must prepare before performing each experiment. The rationale for the experimental setup is very important concept that many students find confusing. In this section, you should explain how the methods that you are going to use in the class allow you to achieve the objective of the lab. Typically, you should show how the technique you will use allows study of the property of interest in your system. You should also offer a plan to interpret your data and predict at least one reasonable outcome. Please note that this section is not a copy of your lab manual's background section nor a place to repeat detailed experimental procedures but involves thoughtful synthesis of all the information available to you.

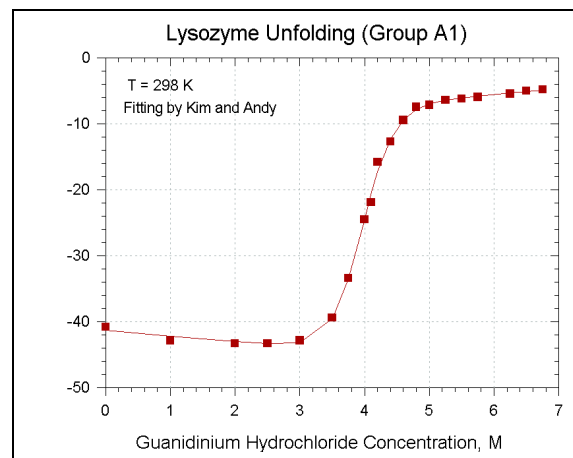
Prelab questions

The prelab questions are designed to help you to understand the experiment that you are doing. Answer your prelab questions directly into your notebook or type them up and attach to your notebook. Make an additional copy of the answers and bring the copy with you to the class. Your TA will collect your prelab including your answers and check them before you can proceed with experiments. Answers to the prelab questions typically contribute about 10% of your total lab report points.

Experiments and group work

You will perform series of experiments in this laboratory and complete several computer assignments. Because of the large number of students and limited resources, students will work in groups to perform the experiments. Some computer assignments (allantoin conformational analysis, statistical data analysis project) will be performed independently. In order to best use the available time, all the students in one section are assigned into one of the four groups when performing other experiments. The schedule outlines experiments that each group does on a particular day.

Students are allowed, but not required, to work with students in their assigned group while performing statistical data analysis of experimental data in protein unfolding, ligand binding, and enzyme kinetics projects. In the case of group analysis, each printed or electronic copy of a data plot should carry names of all the students involved in the data analysis. All other data analysis should be carried out independently.



Quizzes

There will be ten quizzes (listed below) in this lab. The quiz is normally taken on the day of the experiment. All quiz scores count; you can earn up to 100 points

- 1) Conformational analysis and structure optimization methods (allantoin I)
- 2) Monte Carlo and molecular dynamics computer simulation methods (allantoin II)
- 3) Nuclear magnetic resonance spectroscopy (allantoin III)
- 4) Circular dichroism spectroscopy and protein folding
- 5) UV-Vis spectrophotometry and ligand binding equilibrium
- 6) Enzyme kinetics: two-substrate kinetics
- 7) Enzyme kinetics: enzyme inhibition
- 8) Protein mass spectrometry
- 9) Protein crystallization
- 10) X-ray diffraction analysis in biochemistry

Exams

There will be two exams. The first will be on structure determination by NMR and modeling, CD measurements of protein unfolding, and enzyme kinetics. The second will cover mass spectrometry, ligand binding by UV-Vis spectrophotometry, and protein crystallization. The purpose of the exams is to evaluate (1) your understanding of the theory behind the technique and (2) your ability to analyze the physical meaning of the data obtained. Each exam is worth 50 points, and the total maximum from two exams is 100.

Poster presentation

There will be a poster presentation at the end of the quarter. Currently, the poster session is planned for noon to 2 PM for Friday, June 7th. The poster presentation is worth 20 points. Each group will present one poster about one of the experiments performed in this course.

Discussion Sections

There are days in which students participate in the discussion sections. During the discussion sections, we review the background of some experiments and explain relevant concepts and approaches. Successful participation in discussions requires that you know well mathematics, organic chemistry, physical chemistry, and general biochemistry. Each student can earn up to 5 points from each discussion (up to maximum 35) by carrying out mathematical derivations, explaining concepts or solving problems on the board. Please note that students in smaller section have a certain advantage as they have more opportunities to earn their points; this is intentional. The discussion sections are

- 1) Quantum mechanics and molecular mechanics
- 2) Monte Carlo and molecular dynamics simulations
- 3) Nuclear magnetic resonance spectrometry
- 4) Single-substrate enzyme kinetics
- 5) Multi-substrate enzyme kinetics
- 6) Enzyme inhibition (over two days)
- 7) Statistical data analysis
- 8) Peptides and proteins: structure, folding, and ligand binding thermodynamics
- 9) Protein crystallography

Grading

Your grade will be based on the number of points you earn out of 470 points possible. There are six lab write-ups. You may make up failed experiments during the last two weeks. The points for each experiment are as follows:

Allantoin conformational analysis: computer modeling and NMR	40
Protein unfolding monitored by circular dichroism (unfolding of lysozyme)	30
Ligand binding by lysozyme: determination of the equilibrium constant	30
Electrospray ionization mass spectrometry characterization of proteins	30
Enzyme kinetics: glyceraldehyde-3-phosphate dehydrogenase	40
Protein crystallography	30
Lab work ethics/cleanliness	15
Poster presentation	20
Total Lab	235
Exams	100
Quizzes	100
Participation in discussion sections	35
Total Theory	235
Grand Total	470

Grading will be based on the curve and I anticipate that students who collect 67% or more of the possible points will receive grade of C or better. The percentage of students who have received A- or better has been around 20% in the past. The percentage of students who have received grade C- or lower in this course has been around 10–15% in the past.

Wishing you the best success in this course

-- Kalju --