



## ***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:

<http://www.chem.ucsb.edu/~kalju/chem112L>. 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 33% 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 21% 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://hep.ucsb.edu/people/hnn/conduct/disq.html>

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 rules<sup>1</sup> 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 four 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. No shorts are allowed, and long pants and long sleeves are recommended.
- 4) Label all solutions that you prepare clearly. If the solution is in a container larger than 5 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.

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<sup>1</sup> 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  $\mu$ 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 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 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 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

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. Please consult the “Experiment design, scientific data analysis and presentation” handout from Chem 110L for tips on how to prepare figures. Each report should include the following (percentage of grade shown in parentheses):

- title page giving the name of the experiment, your name, and grading rubric
- 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 %)
- an appendix with all the raw data that were collected, and all calculations that you performed

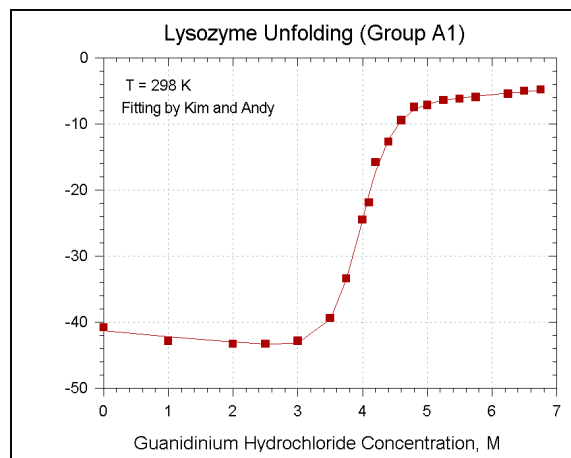
The objective of the experiment, the rationale for the experimental set-up, concise summary of the procedure, and answers to your prelab questions constitute the *prelab* that you must prepare before performing each experiment. The rationale for the experimental setup is very important concept that many 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 but make an additional carbon copy (or photocopy) of the answers and bring the copy with you to the class. Your TA will check your prelab and collect the carbon copy with your answers to the prelab questions. He will also tell you if any of your answers to prelab questions were wrong. You may correct your wrong answers and get up to 25% of credit on the wrong answer when you turn your notebook in for final grading. Please mark clearly the corrected answer. 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 in this lab:

- 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

The quiz is normally taken on the day of the experiment. All quiz scores count; you can earn up to 100 points.

### **Exams**

There will be two exams. The first will be on protein folding by structure determination by NMR and modeling, CD measurements of protein unfolding and ligand binding equilibria. The second will cover mass spectrometry, enzyme kinetics, 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 6<sup>th</sup>. 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 30) 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) Statistical data analysis
- 4) Peptides and proteins: structure, folding, and ligand binding thermodynamics
- 5) Single-substrate enzyme kinetics
- 6) Multi-substrate enzyme kinetics
- 7) Enzyme inhibition

## Grading

Your grade will be based on the number of points you earn out of 500 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	50
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	40
Enzyme kinetics: glyceraldehyde-3-phosphate dehydrogenase	40
Protein crystallography	40
Lab work ethics/cleanliness	20
<b>Total Lab</b>	<b>250</b>
Exams	100
Quizzes	100
Poster presentation	20
Participation in discussion sections	30
<b>Total Theory</b>	<b>250</b>
<b>Grand Total</b>	<b>500</b>

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 25% in the past. The percentage of students who have received grade C- or lower in this course has been less than 10% in past.

*Wishing you the best success in this course*  
*-- Kalju --*

SCHEDULE FOR SECTION 1 (MW 2PM)

**Week 1**

March 31 Introduction to the course. [Discussion of quantum mechanics and molecular mechanics \(Dr. Kalju Kahn\)](#).

April 2 [Discussions of Monte Carlo and Molecular Dynamics \(Dr. Kalju Kahn\)](#)

**All groups:** Allantoin Part I: Conformational analysis in the gas phase.

**Week 2**

April 7 **All groups:** [NMR Lecture \(Dr. Kalju Kahn\)](#)

**All groups:** Allantoin Part II: Monte Carlo simulation in solution

April 9 **Group A:** Allantoin Part III: NMR data acquisition (meet 2:00 in PSB-N 2619)

**Group B:** Allantoin Part III: NMR data acquisition (meet 4:00 in PSB-N 2619)

**Week 3**

April 14 **All groups:** [Demonstration of the CD spectrophotometer at 5 PM](#)

**All groups:** Scientific Data Analysis Tutorial, (Phelps 1526: Miramar) 2:00-4:50

**Group A1:** Preparation of solutions for circular dichroism

April 16 **All groups:** [Discussion of UV/Vis and Ligand Binding](#)

**Group A1:** Circular dichroism study of protein folding

**Group B:** UV/VIS binding study of NAG to lysozyme

**Group A2:** Time for independent study

April 18 **Group B1:** Preparation of solutions for circular dichroism

**Week 4**

April 21 **All Groups:** *Data Analysis Assignment, "Conformational Analysis of Allantoin" project due.*

**Group A:** UV/VIS binding study of NAG to lysozyme

**Group B1:** Circular dichroism study of protein folding

**Group B2:** Preparation of solutions for circular dichroism / time for independent study

April 23 **Group B2:** Circular dichroism study of protein folding

**Group A:** Time for independent study

April 25 **Group A2:** Preparation of solutions for circular dichroism

**Week 5**

April 28 **All Groups:** [Lecture and Discussion: Enzyme Kinetics I \(Dr. Kalju Kahn\)](#)

**Group A2:** Circular dichroism study of protein folding

**Group B:** Enzyme kinetics: Multi-substrate kinetics with GAPDH

Apr 30 **All Groups:** [Lecture and Discussion: Enzyme Kinetics II \(Dr. Kalju Kahn\)](#)

**Group A:** Enzyme kinetics: Multi-substrate kinetics with GAPDH

**All Groups:** *"Ligand Binding to Lysozyme" project due.*

**Week 6**

May 5 **All Groups:** [Lecture and Discussion: Enzyme Kinetics III \(Dr. Kalju Kahn\)](#)

**Group A:** Enzyme kinetics: Inhibition of GAPDH

May 7 **All Groups:** [Mass spectrometry lecture](#)

**Group B:** Enzyme kinetics: Inhibition of GAPDH

**Week 7**

May 12 **All Groups: “Circular Dichroism and Protein Folding” project due**

**All Groups:** Protein crystallography: set up crystallization trials

May 14 **All Groups: *First Midterm (Modeling, NMR, CD, UV-Vis, Ligand Binding, Folding)***

**Week 8**

May 19 **Group A:** Protein mass spectrometry

**Group B:** Protein crystallography: microscopic analysis of crystals

May 21 **All Groups: “Enzyme Kinetics” project due**

**Group A:** Protein crystallography: microscopic analysis of crystals

**Group B:** Protein mass spectrometry

**Week 9**

May 26 **Memorial Day Holiday**

May 28 **All Groups: “Mass Spectrometry” project due.**

**All Groups:** Protein crystallography: analysis of diffraction patterns

**Week 9**

June 2 **All Groups: “Protein Crystallography” project due**

**All groups:** Discussion: How to prepare for the poster session (Kahn)

June 4 **Make-up / clean-up day**

June 6 **Class will meet on June 6 (Friday) for the poster presentation (Noon ?)**

***Second midterm (MS, Enzyme Kinetics, Protein Crystallography) will be at the time allocated by the registrar for the final***

## SCHEDULE FOR SECTION 2 (TR 6PM)

### **Week 1**

April 1 Introduction to the course.  
[Discussion of quantum mechanics and molecular mechanics \(Dr. Kalju Kahn\).](#)

April 3 [Discussions of Monte Carlo and Molecular Dynamics \(Dr. Kalju Kahn\)](#)  
**All groups:** Allantoin Part I: Conformational analysis in the gas phase.

### **Week 2**

April 8 **All groups:** [NMR Lecture \(Dr. Kalju Kahn\)](#)  
**All groups:** Allantoin Part II: Monte Carlo simulation in solution

April 10 **Group A:** Allantoin Part III: NMR data acquisition (meet 6:00 in PSB-N 2619)  
**Group B:** Allantoin Part III: NMR data acquisition (meet 7:30 in PSB-N 2619)

### **Week 3**

April 15 **All groups:** [Demonstration of the CD spectrophotometer at 6:40 PM](#)  
**All groups:** [Scientific Data Analysis Tutorial/ Discussion, \(Phelps 1525: Mesa\) 7:00-9:50](#)  
**Group A1:** Preparation of solutions for circular dichroism

April 17 **All groups:** [Discussion of UV/Vis and Ligand Binding](#)  
**Group A1:** Circular dichroism study of protein folding  
**Group B:** UV/VIS binding study of NAG to lysozyme  
**Group A2:** Time for independent study

### **Week 4**

April 21 **Group B1:** Preparation of solutions for circular dichroism

April 22 **All Groups:** ***Data analysis assignment, "Conformational Analysis of Allantoin" project due.***  
**Group A:** UV/VIS binding study of NAG to lysozyme  
**Group B1:** Circular dichroism study of protein folding  
**Group B2:** Preparation of solutions for circular dichroism / time for independent study

April 24 **Group B2:** Circular dichroism study of protein folding  
**Group A:** Time for independent study

April 25 **Group A2:** Preparation of solutions for circular dichroism

### **Week 5**

April 29 **All Groups:** [Lecture and Discussion: Enzyme Kinetics I \(Dr. Kalju Kahn\)](#)  
**Group A2:** Circular dichroism study of protein folding  
**Group B:** Enzyme kinetics: Multi-substrate kinetics with GAPDH

May 1 **All Groups:** [Lecture and Discussion: Enzyme Kinetics II \(Dr. Kalju Kahn\)](#)  
**Group A:** Enzyme kinetics: Multi-substrate kinetics with GAPDH  
**All Groups:** ***"Ligand Binding to Lysozyme" project due.***

### **Week 6**

May 6 **All Groups:** [Lecture and Discussion: Enzyme Kinetics III \(Dr. Kalju Kahn\)](#)  
**Group A:** Enzyme kinetics: Inhibition of GAPDH

May 8 **All Groups:** [Mass spectrometry lecture](#)  
**Group B:** Enzyme kinetics: Inhibition of GAPDH

**Week 7**

May 13 **All groups: “Circular Dichroism and Protein Folding” project due**

**All Groups:** Protein crystallography: set up crystallization trials

May 15 **All Groups: *First Midterm (Modeling, NMR, CD, UV-Vis, Ligand Binding, Folding)***

**Week 8**

May 20 **Group A:** Protein mass spectrometry

**Group B:** Protein crystallography: microscopic analysis of crystals

May 22 **All Groups: “Enzyme Kinetics” project due**

**Group A:** Protein crystallography: microscopic analysis of crystals

**Group B:** Protein mass spectrometry

**Week 9**

May 27 **All Groups:** Protein crystallography: analysis of diffraction patterns

May 28 **All Groups: “Mass Spectrometry” project due.**

**Week 9**

June 3 **All Groups: “Protein Crystallography” project due**

**All groups:** Discussion: How to prepare for the poster session (Kahn)

June 5 Make-up / clean-up day

June 6 **Class will meet on June 6 (Friday) for the poster presentation (Noon ?)**

***Second midterm (MS, Enzyme Kinetics, Protein Crystallography) will be at the time allocated by the registrar for the final***