CHEM 4242: TECHNIQUES IN BIOCHEMISTRY

SPRING 2016
Wednesday/Thursday 1:00 P.M. - 5:00 P.M.

Instructor
Dr. Jongyun Heo
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Office hours: 12:30 P.M. – 1:30 P.M. Tuesday & Thursday

Wednesday section: TA: Paromita Deb
Office: CPB 319
Office Hour 10:00 A.M. - 12:00 P.M. (Wednesday)
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Thursday section: TA: Hope Gloria Umutesi
Office CPB 319
Office hour: 10:00 A.M. - 12:00 P.M. (Thursday)
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Lecture: 315 Science Hall; Time: 1:00 - 1:50 P.M
Laboratory: 210 CPB; Time: 2:00 - 4:50 P.M.

***TEXT BOOK*:** "Modern Experimental Biochemistry" Third Edition by Rodney Boyer as well as the handouts

***COURSE OVERVIEW AND OBJECTIVES*:** Welcome to biochemistry laboratory! This practical course is intended to give you an opportunity to learn the basic techniques routinely used in biochemistry and molecular biology. The students will be introduced to the theory that serves the basis for these techniques. It will include discussion of the theoretical principles, practical details and applications of the key experimental techniques, including those that have led to the emergence of the new disciplines of genomics, proteomics and bioinformatics. The students should be able to perform the biochemical experiments independently and interpret the lab results. Each experiment will cover the principles of experimental design and the statistical analysis of quantitative analytical data. To enhance student understanding of each topic, in-text worked examples are included in each lab. The textbook "Modern Experimental Biochemistry" by Rodney Boyer as well as the handouts will be given for each lab one week prior and will be discussed during the lecture hour and lab. Practical applications of all commonly used analytical techniques will be discussed.

By the end of the course you should be *familiar with a number of common biochemical techniques:* use of pipettes and other biochemistry related instruments, making buffers, handing techniques for protein and DNA, methods of protein determination; analysis of enzyme kinetics; methods of protein purification; electrophoresis (SDS-PAGE and Agarose gel), plasmid purification, restriction analysis, and polymerase chain reaction (PCR).

LAB REPORT: To be prepared for each laboratory session. Submit the handwritten lab report at the assigned dates (please see Table).

***LAB REPORT FORMAT*: *M*UST BE HAND-WRITTEN and NEAT**. Points will be deducted from late reports. ***Late reports will not be accepted after the graded reports for that experiment have been handed back to the class*.**

1. Title of the Experiment.

2. Introduction, Objective and principle: (Total should not exceed more than 1 page) The purpose of the experiment should be clearly stated in no more than five lines. You may draw some figures if needed.

3. Reagents and Procedure (one page).

4. Results and Calculations: How you prepared your solutions/buffer (show calculation involved in making the mentioned concentration). Also write your observations which you note while doing the experiment (2 pages).

5. Discussion and Conclusions: This section should include your interpretations of the data and draw conclusions. You should connect your results with those that were expected, to those of others and/or to what is already known. If you obtain unexpected results, provide possible explanations. This section should be the most detailed and include interpretation of ALL of your data. **The discussion should be no longer than 2 pages.**

Answers to the questions given in the time-table for each lab should be submitted along with the lab report.

***GRADING POLICY*:**
Each lab (lab 1 through 6) carries 10 points for a total of 60% of the grade.

Student must write the *pre-lab* section and bring it before start of the lab to the TA and get it signed. The pre-lab section should clearly indicate 1) title of the experiment; 2) introduction and objective; and 3) reagents and procedure.

Bringing the pre-lab section and getting it initialed by the TA is very important for getting the pre-lab grade.

10 points of a lab will be counted as: 2 points for the prelab, 6 points for the Results and Calculations and 2 points for the Discussion and Conclusions.

Each quiz (quiz 1 through 6) carries 4 points for a total of 24% of the grade. The final exam carries 16% of the grade.

Quizzes: Quizzes will be given as noted in the timetable. The quizzes will be given at the lecture portion/lab portion of the course and will be based on the readings and problems given in the timetable.

Attendance/Participation: You are expected to participate in each lab session. **There will be no "make-up" labs.** Please see the instructor if you miss lab. Points will be deducted if you are not fully participating in the experiment and/or clean-up; for example,

if your partner does most of the work or if you are unable to perform basic lab techniques.

BE ON TIME!! Each lab period will begin with an important short lecture. In addition, any changes in procedures are described and required supplies are distributed at the beginning of the lab period.

Cleanliness, etc. The students in the lab class are responsible for keeping the equipment and laboratory neat and tidy. Glassware will be cleaned by students in the laboratory before leaving.

***STUDENT RESPONSIBILITIES*:**
You will need to purchase a copy of the textbook "Modern Experimental Biochemistry" by Rodney Boyer, Third Edition in the bookstore. You will need to use a computer and/or a calculator and graph paper to calculate and analyze data for lab reports.

MSDS: Guidelines for working safely in Chemistry laboratories (MSDS). Look up for hazards and handling procedures for chemicals you use in the lab.

Time Table:

Date	Subject of the Laboratory	Chapters from Rodney Boyer	Problems/reading	Quiz dates/Due dates for lab report
Jan 20-21	Check-in	Handout for Lab 1		
Jan 27-28	Lab 1:Introduction to electrophoresis: SDS-PAGE	Handout for Lab 2 Chapter 4 pp.111-140	pp. 138-139 #1,4,5,8,9 &10	Quiz 1 (on Lab 1)
Feb 03-05	Lab 2: Using gel filtration to study ligand-protein interaction (Part I)	Experiment 3 pp.243-256	pp.254 #5,6,9 &10	Report of Lab 1 Quiz 2 (Lab 2)
Feb 10-11	Lab 2: Using gel filtration to study ligand-protein interaction (Part II)	Handout for Lab 3		
Feb 17-18	Lab 3: Expression and purification of plasmid DNA (Part I)	Experiment 14 and 15 pp. 415-442	pp.428 #4,6,7,8,9 & 10; pp. 440-441 #1,2,4,7,8 &10	Report of Lab 2 Quiz 3 (Lab 3)
Feb 24-25	Lab 3: The action of endonucleases on plasmid DNA (Part II)	Handout for Lab 4		
Mar 02-03	Lab 4: In vitro amplification of DNA by PCR (Part I) Include gradient PCR			Quiz 4 (Lab 4) Report of Lab 3
Mar 9-10	Lab 4: In vitro amplification of DNA by PCR (Part II)	Handout for Lab 5		
Mar 14-18	**Spring break**			
Mar 23-24	Lab 5: Kinetic analysis of tyrosinase (Part I)	Experiment 5 pp. 279-301	pp. 299-300 #2,7,8,9 &10	Quiz 5 (Lab 5) Report of Lab 4
Mar 30-31	Lab 5: Kinetic analysis of tyrosinase (Part II)			
April 06-07	Lab 6: Isolation and Characterization of Bovine milk α-lactalbumin Part I	Experiment 4 pp.257-277	pp.55 #1 & 4 pp. 207-208 #1,2,3,4 &9	Quiz 6 (Lab 6) Report of Lab 5
April 13-14	Lab 6: Isolation and Characterization of Bovine milk α-lactalbumin Part II		pp.107 #7	
April 20-21	Lab 6: Isolation and Characterization of Bovine milk α-lactalbumin Part III	Handout for Computer Lab	pp. 168-169 #2 &6	Homework
April 27-28	Lab 7: Computer Lab	Take Home Final Exam		Report of Lab 6
May 04-05	Check-Out			Final exam & Report of Lab 7 due

CHEM 4242: LABORATORY TECHNIQUES IN BIOCHEMISTRY
Syllabus and Course Information

Lab 1: Introduction to Electrophoresis: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of proteins
The objectives of the lab are:

- The students will be introduced to electrophoresis; a technique used in biochemistry and molecular biology to separate proteins according to their size (length of polypeptide chain or molecular weight).
- The students will learn to determine the molecular weight of unknown proteins by comparing the mobility of the unknown protein with the mobility of proteins of known molecular weight.

Lab 2: Using gel filtration to study Ligand-Protein Interactions
This lab has been designed to study the protein-ligand Interactions- The dynamics of protein-ligand interactions will be explored with the binding of dye phenol red to bovine serum albumin. The technique of gel filtration will be used for the molecular separation of ligand from the complex because the ligand-protein complex often has a molecular weight much greater than that of the free ligand, which is usually a smaller molecule. After separation, absorbance measurements will be used to quantify the ligand-protein complex and free ligand concentration. Data will be analyzed in order to construct the binding curves.

Lab 3(I): Expression and purification of DNA
The objectives of the lab are to purify plasmid DNA from bacteria. The method involves:

- Growth of the bacterial culture.
- Harvesting and lysis of the bacteria.
- Purification of plasmid DNA using anion-exchange resin; and quantification of the purity and yield of plasmid DNA. The students will be introduced to agarose gel electrophoresis and to calculate the size and purity of the plasmid DNA.

Lab 3(II): The action of endonucleases (restriction enzymes) on plasmid DNA
Restriction enzymes are used extensively in nucleic acid chemistry. These enzymes reproducibly cleave DNA at specific sites. This feature provides a convenient way to break large DNA molecules into smaller fragments for subsequent analysis and manipulations. It is unlikely that the set of fragments will be the same for two different DNA molecules, so fragmentation pattern is unique and can be considered a “fingerprint” of the DNA substrate.

Lab 4: In vitro amplification of DNA by PCR
Students will be introduced to a technique Polymerase Chain Reaction (PCR), a technique proved to be a boom to the molecular biology. PCR is a process based on a specialized polymerase enzyme, which can synthesize a complementary strand to a given DNA strand in a mixture containing the 4 DNA

bases and 2 DNA fragments (primers, each about 20 bases long) flanking the target sequence. The mixture is heated to separate the strands of double-stranded DNA containing the target sequence and then cooled to allow (1) the primers to find and bind to their complementary sequences on the separated strands and (2) the polymerase to extend the primers into new complementary strands. Repeated heating and cooling cycles multiply the target DNA exponentially, since each new double strand separates to become two templates for further synthesis. In about 1 hour, 20 PCR cycles can amplify the target by about million fold.

Lab 5: Kinetic analysis of Tyrosinase

In this experiment, students will determine the kinetic properties of Tyrosinase, a copper-containing oxidoreducatase, which catalyzes the orthohydroxylation of monophenol substrates and aerobic oxidation of catechols. The enzyme will be assayed by monitoring the oxidation of 3,4-dihydrophenylalanine (dopa) to red-colored dopachrome. Kinetic parameters K_M and V_{max} will be evaluated using Line weaver-Burk or direct linear plots. Two stereoisomer, L-dopa and D-dopa, will be tested and compared as substrates. Inhibition of tyrosinase by thiourea and cinnamic acid will be studied. This experiment has been divided into two parts.

Lab 6: Isolation and characterization of Bovine milk α-lactalbumin

In this lab, students will learn:

- Isolation of α-lactalbumin from bovine milk.
- Separation of α-latalbumin and β-lactalbumin from other immunoglobulins.
- Separation of α-actalbumin from β-lactalbumin.
- Purification of α -lactalbumin using gel filtration and affinity chromatography.
- Quantification of α-lactalbumin by Bradford assay, SDS-PAGE and UV-Vis spectroscopy.
- Determination of Molar absorption coefficient of α -lactalbumin.

The lab has been divided into three parts.

Lab 7: Computer lab (to explore the three-dimensional structure of proteins)

Protein Explorer (http://www.proteinexplorer.org) enables students to visualize macromolecular structures easily. It is very powerful; in addition to basic macromolecular visualization capabilities, it offers one-click visualization of interfaces between moieties ('contacts'), cation-pi interactions and salt bridges, as well as easy-to-use routines to visualize regions of conservation in three-dimensional protein structures based on multiple sequence alignments.