Laboratory in Biochemical Techniques (NSC425) Fall 2007

Instructors Todd Smith (Sci 110), Allison Turner (Sci 206)

Classroom	Science 112
Day & time	Thursday 1:30-4:50
Readings	Various lab protocols as hand-outs

Course Description

This laboratory will be an introduction to techniques commonly used by biochemists, and must be taken in conjunction with Biochemistry of the Cell. Your work in the laboratory will focus on a semester-long investigation of a protein extracted from papaya fruit. This project will allow you to perform your own biochemistry research project in which you will employ the principles of chemistry and biochemistry that we study in the classroom.

The protein you will investigate, papain, is a protease – it degrades proteins. This particular protein is purportedly used in products such as meat tenderizer and laundry detergents. Your goal is to determine if the papain you isolate from fresh (or dried) papaya is the same as that contained in commercial products such a meat tenderizer. To answer this question we will begin with basic laboratory procedures such as preparing reagents, chromatography, and performing a protein assay. We will then explore techniques for studying the activity of enzymes, and methods for separating proteins, such as one and two-dimensional electrophoresis. Finally we will employ methods for the identification of specific proteins using immuno-staining, and a phenomenally sensitive technique for quantifying a specific protein in solution, the enzyme-linked immuno-sorbent assay (ELISA). Throughout this semester-long project you will also learn about the procedures for data acquisition and analysis that will allow you to draw meaningful conclusions from your results.

Grading Policy

Students are expected to attend all labs and to complete all reading assignments. There will be two laboratory reports due over the course of the semester, each of which will describe the current status of your research project. Each report is worth 30 points. You will also keep a lab notebook (see below), which will be evaluated at the end of the semester, and will be worth 20 points. The final portion of your grade (10 points) will be based on your preparation for and performance in the lab. The grade received in the course will be the ratio of points earned over points possible: 90% & above = A, 80-89% = B, 70-79% = C, 60-69% = D. Diligent and conscientious participation in lab and on assignments will boost borderline grades to the higher grade. If extenuating circumstances will prevent you from coming to lab or completing a lab report notify me as soon as possible so that we can make alternative arrangements for administering the exam.

Laboratory Notebooks

For this course you will need a dedicated laboratory notebook. For each laboratory session you should record the purpose of the lab, what you did in the lab, and what you found. Your notebook is a place to keep detailed notes of these activities in a format that

will allow you to make sense of your lab activities at a later date, e.g. when writing a lab report at 3 a.m.

Prerequisites:	General Chemistry I & II
Corequisite:	Biochemistry of the Cell

Schedule for the semester

Date	Lab	Торіс	Assignment
9/5	0	Intro session	
9/13	1	Laboratory safety; Size-exclusion chromatography & protein purification – papain from papaya	Bring a lab notebook
9/20	2	Chromatography & protein purification continued	
9/27	3	Protein assay & standard curve; data organization, analysis & presentation	
10/4	4	Open research day	
10/11	5	Enzyme assays: measuring activity of the enzyme papain	Lab report 1
10/18	6	Control of papain activity by enzyme inhibition	
10/25		Protein electrophoresis I: iso-electric focusing	
11/1	7	Protein electrophoresis I - continued	Lab notebooks due
11/8	8	Protein electrophoresis II: the second dimension	
11/15	9	Gel analysis: staining and calculating protein molecular weights	
11/22	10	Thanksgiving – no lab	
11/29	11	Immunostaining: using antibodies to identify and quantify proteins	Lab report 2
12/6	12	Immunostaining – continued Lab clean-up	Lab notebooks due