PROTEIN PURIFICATION AND ANALYSIS (BIO 349)
SPRING 2009

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Office Hours: Monday 10-12, Wednesday 10-12, and by appointment

Prerequisites
BIO 231 and junior or senior status

Required Texts
Ninfa and Ballou—*Fundamental Laboratory Approaches for Biochemistry and Biotechnology*

Course Information
Course Description: A 7-week laboratory-intensive course covering the methodology of protein purification using nickel affinity columns, quantification of protein concentration, and protein analysis via PAGE. Various methods of detection will be used including coomassie blue stain and western blot.

Goals: The goal of the course is to prepare you for either graduate studies in molecular biology or a technician position in a research lab. The lecture portion of this course will help you to better understand methods used to purify and analyze proteins in the molecular biology laboratory. The laboratory portion of the course will allow you to experience some of those methods as we isolate, purify, and examine two different proteins. You will also learn how to interpret your data through the use of biotechnology.

Objectives: At the completion of this course, students will be a to:
1) Analyze Protein Structure using computer imaging.
2) Understand both denaturing and non-denaturing polyacrylamide gel electrophoresis (PAGE) as well as various methods of protein detection used in conjunction with PAGE.
3) Understand various methods of protein purification.
4) Assess the results of protein purification through quantification and characterization of enzyme activity.

Outcomes and Assessment:
1) Students will be able to use computer software to analyze protein structure. This ability will be assessed through a written assignment that requires the use of RasMol to analyze the structure of □ amylase.
2) Students with prepare both denaturing and non-denaturing polyacrylamide gels, run protein samples on these gels, and use Coomassie Brilliant Blue Dye, Fast Red TR/napthol AS-MX stain, and immunoblotting (Western blotting) to examine the protein bands on these gel. Students will be assessed by both class participation and through data in their lab notebooks.

3) Students will have a general understanding of various types of chromatography, ways of "salting-out" proteins, and precipitating proteins for purification and concentration. Students will use dialysis, ammonium sufate precipitation, and DEAE-cellulose column chromatography to purify alkaline phosphatase. This information will be assessed in a final examination in which students will be asked to recall details from information presented in lectures. Additionally, application of the techniques will be assessed by class participation and examining lab notebook entries.
4) Students will learn four basic methods used for quantifying proteins in a sample and use the Bradford Method to examine their samples. Students will also examine the enzymatic activity of their samples to determine percent yield. They will be asked to use obtained data to draw conclusions about their methods of purification. Students will be assessed through the data interpretation section of their laboratory notebooks and in application of information in the final exam.

Grading

1. Lab Notebook (20%): You will be expected to keep an updated lab notebook in which you will demonstrate your knowledge of the projects, the work that you did both in and out of class, and the analysis of your data. At the front of this notebook please write your name and make a table of contents. Each following page should be dated. As this course will involve two separate projects, they should be detailed in two separate sections of your lab notebook. In each section you should include information about:

1. The purpose of the project
2. The materials used
3. What protocol was used, including where to find it (text and page number) and any modifications you made to it
4. Results of the experiment (including data)
5. Interpretations of the experiment and any conclusions that you could draw from the project
6. Suggestions for next time you conduct a similar project

2. Protein Explorer Questions (10%): You will use a tutorial program to learn how Protein Explorer is used to examine the structure of proteins. You will answer questions that go along with this project.

3. Class Participation (10%): Class participation is obviously crucial to laboratory performance. I expect every lab group member to attend every class and lab group meeting and participate equally in all exercises. Unexcused absences will count against this portion of your grade.

4. **Lab Report (25%):** You will write a final lab report in the format of a scientific paper that describes your study on various methods for purifying alkaline phosphatase. Your report should contain all of the components of a scientific paper (Title, Abstract, Introduction, Methods, Results, Discussion, Literature Cited). Your textbook describes each of these section s (p 47-48), so refer to it for further information. In your report I do expect you to introduce the project in the Introduction section, naming the organism used and describing the protein of interest. The majority of the information can be obtain from your textbook (make sure that you include it in your literature cited section). The methods section should briefly describe the varousl

techniques that you used for purification. The focus of your paper should be on the results and discussion sections—you should describe the best method for purifying alkaline phosphatase and use your data to support your conclusion.

4. Final Exam (35%): We will have a lab final after the last day of class during which you may demonstrate your mastery of the material presenting in this class.

Grade Tally:

Lab Notebook		20%
Protein Explorer		10%
Course Participation		10%
Scientific Paper	25%	
Final Exam		35%

Grading Scale:

92.0 – 100	A	78.0 – 79.9	C+
90.0 - 91.9	A-	72.0 – 77.9	C
88.0 – 89.9	B+	70.0 – 71.9	C-
82.0 – 87.9	B	60.0 – 69.9	D
80.0 – 81.9	B-	less than 59.9	F

Academic Philosophies

Honor Code: Students are expected to comply with the Cedar Crest College Honor Code as stated in the Catalog.

Classroom Protocol: Students are expected to comply with the Cedar Crest College Classroom Protocol Code as stated in the Catalog.

Plagiarism: Students are expected to comply with the Cedar Crest policy on plagiarism. Cases of plagiarism, whether deliberate or accidental, will not be tolerated and will result in an "F" for the given assignment.

Learning Disabilities: Students with documented disabilities who may need academic accommodations should discuss these needs with me during the first two weeks of class. Students with disabilities who wish to request accommodations should contact the Advising Center.

Attendance: You are expected to attend and actively participate in all lectures and laboratory exercises. I expect you to arrive to class in a timely manner. It is your responsibility to inform me of planned absences and it is your responsibility to obtain any assignments, handouts, etc. Absences on days of exams, presentations, or the collection of assignments will have to be approved by the Dean of Students. If the Dean of Students does not approve the absence, you will receive a zero for that portion of your grade. Be warned: most molecular biology experiments do not fit neatly into a three-hour time period. You will be expected to come into lab on other days and times to continue your work. Make arrangements with your lab partner to pick a time best suited to your schedules. Realize, however, that I am a *morning* person, so if you choose to come to lab late in the evening/night, I will not be available to help you.

General Course Information

We will cover Chapters 1-7

- Chapter 7: Purification Protocols
- Chapter 5: Gel Electrophoresis Protocols (SDS-PAGE p 147, Non-denaturing p 153 + Handout)
- Chapter 3: Quantification Protocol (Bradford, p 87)
- Chapter 7: Enzyme Assay Protocol (Fixed-Time, p 194)
- Handout for Gel Staining (Coomasie Blue)
- Handout for Immunoblotting
- Handout for Data Collection

Lab Procedure (Differs from described in the book)

- Start with 20ml (double all added components for Part I, p 180-182)
- After day one dialysis, remove 5ml for heat denaturation (p 183-184), remove 5ml for ammonium sulfate precipitation (p 184-186), remove 5ml for column purification (p 187-189), remainder is analyzed as un purified sample (4 samples total for analysis)
- Analysis 1: SDS-PAGE and non-denaturing PAGE (p 147, 153, HO)
    - Coomassie Blue of SDS-PAGE (HO)
    - Coomassie Blue of non-denaturing PAGE (HO)
    - Enzyme Assay of non-denaturing PAGE (p 154)
    - Immunoblot of SDS-PAGE (HO)
- Analysis 2: Enzyme Assay (p 194)
- Analysis 3: Bradford Assay (p 87)
- Completion of Data Table
- Written Report of Results (due at final exam)

COURSE SCHEDULE

Date	Exercises	Reading
January 22	Prepare samples (Ninfa p. 179-184) Dialysis (Ninfa p. 182) Make sure you properly store your samples on Friday!!! Group A: pour protein gels (Ninfa p. 147-150 and handout)	Chapters 1 and 7
January 29	Purification Steps: Set aside 5 ml of pre-purification sample Heat Denaturation of 5ml (Nifa p. 183-184) Ammonium sulfate precipitation of 5 ml (Ninfa p. 184-186) DEAE-cellulose column chromatography of 5 ml (Ninfa p. 186-189) Protein Analysis: Enzyme Assay (Ninfa p.194-195) Bradford Assay (Ninfa p. 87)	Chapters 6 and 4
February 5	Group A: Non-denaturing PAGE (Ninfa p. 152-154) Blot gel (handout) Gel stains (Ninfa p. 154) Group B: Pour protein gels (Ninfa p. 147-150 and handout) Continue Purification and Analysis Steps Group C: Protein Explorer Demo (on-line)	Group A: Chapter 5 Group B: Chapters 2 and 3 Group C: Chapter 12
February 12	Group A: Protein Explorer Demo (on-line) Group B: Non-denaturing PAGE (Ninfa p. 152-154) Blot gel (handout) Gel stains (Ninfa p. 154) Group C: Pour protein gels (Ninfa p. 147-150 and handout) Continue Purification and Analysis Steps	Group A: Chapter 12 Group B: Chapter 5 Group C: Chapters 2 and 3
February 19	Group A: Continue Analysis Steps Group B: Protein Explorer Demo (on-line) Group C: Non-denaturing PAGE (Ninfa p. 152-154) Blot gel (handout) Gel stains (Ninfa p. 154)	Group A: Chapters 2 and 3 Group B: Chapter 12 Group C: Chapter 5
February 26	Immunoblot (Handout)	
March 5	Final Exam, Lab Paper Due	