CHEM 4681
Advanced Chemistry Laboratory
Biochemistry Module

Characterization of Enzymes:
Structure, Function, and Stability

Fall 2006

School of Chemistry and Biochemistry
Georgia Institute of Technology

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CHEM 4681: Advanced Chemistry Laboratory
Fall 2006 Biochemistry Module and Goals

Biochemists generally study the chemical and physical characteristics of biological macromolecules, namely proteins, nucleic acids, lipids and carbohydrates. Biological macromolecules can be characterized in a variety of ways. In this module, students will explore fundamental biophysical approaches for characterizing a protein by investigating its structure, function and stability.

There will be several proteins available for students to investigate:

**Alkaline phosphatase** is a ubiquitous enzyme that catalyzes the hydrolysis of phosphate monoesters at high pH (>7) in a wide variety of substrates. Alkaline phosphatase is found in bacteria as well as in higher level organisms in teeth, developing bone, blood plasma, kidney, and the intestine. In humans, alkaline phosphatase levels in blood are correlated with many illnesses including certain forms of anemia.

**Trypsin, Chymotrypsin, and Pepsin** are proteases (proteins that cleave other proteins at specific recognition sites). There are different classes of proteases. Trypsin and Chymotrypsin belong to the class of serine proteases. Serine proteases have three key amino acids in the active site that promote catalysis – serine, histidine and aspartic acid – and are commonly involved in blood clotting, immunity, and other biological functions.

**Tyrosinase** is a metalloenzyme involved in the production of melanin and other pigments from oxidation of the amino acid tyrosine.

There are numerous learning goals for students who pursue this module. Specific technical goals for this project are for students to:

- Characterization of the enzyme via protein concentration assay analysis and gel electrophoresis
- Determination of the kinetic parameters in the presence and absence of an inhibitor
- Determination of the impact of pH on enzyme activity
- Characterization of its unfolding equilibrium
- Correlate experimental results with the 3D structure of the protein

In addition, students will gain some minimal experience in experimental design, critical thinking and practical problem-solving. A considerable portion of the evaluation of student performance will be given to technical writing of one comprehensive, journal-style laboratory report. Students will be given substantial assistance in order to develop a high-quality report from this module.
SECTION 2: Syllabus for Biochemistry Module

GENERAL INFORMATION

Instructor: Mary E. Peek, Ph.D.
Biochemistry Laboratory Coordinator

E-mail: mary.peek@chemistry.gatech.edu (preferred method of contact)
Please include "CHEM 4681" in subject line

Office: Boggs Room 2-71

Course Time: Wednesdays and Fridays, 1:05 - 5:55 pm

Location: Boggs Room 2-10, Biochemistry Teaching Laboratory

LABORATORY POLICIES AND GRADING FOR BIOCHEMISTRY MODULE

The instructor will evaluate student performance in the biochemistry module of the Advanced Chemistry Laboratory based on the following criteria:

40% - Daily Laboratory Performance
40% - Project Report
20% - Laboratory Notebook Maintenance

Final grades for student performance on the Biochemistry module of this course will be given to the coordinator for the course. The coordinator will provide guidelines for final course grading.

Daily Laboratory Performance

• Student performance consists of preparedness for each laboratory session, resourcefulness, creativity, depth of comprehension, and productivity in the laboratory, among other standard indicators of performance.

• Students are expected to have read the laboratory protocol for the day prior to reporting to class.

• Students are responsible for the upkeep of their assigned workstations. Be sure to restock all pipette tip boxes at the end of each class period.

• Be sure to clean all glassware before the end of the class period.

• Keep the balance and instrumentation areas clean and free from clutter.

• Be sure to report any malfunctions in equipment to the instructor.
• All materials stored in the refrigerator, freezers, or at room temperature must be capped and clearly labeled with your name and its contents.

• Follow all safety regulations and encourage others around you to work safely as well.

• Report damaged or broken equipment, supplies or materials to the TA. Students will be responsible for replacing damaged or broken items in the laboratory by paying for the cost via his/her Buzz card. Final grades for the Biochemistry module will not be submitted until all debts have been paid. In extreme cases of negligence, the instructor reserves the right to disallow the student from continuing to perform laboratory experiments until the student is capable of working responsibly in the laboratory; this policy is intended to ensure the safety of the community and to preserve the lifetime of our equipment.

Laboratory Hazards and Safety

Some of the chemicals used in this laboratory are harmful if inhaled or ingested. Follow all safety procedures outlined, including the following:

• Do not allow laboratory chemicals to enter your mouth or small cuts or scratches on your hands.
• Do not inhale powders or vapors.
• Do not eat, drink, or chew gum in the laboratory.
• Do not discard food trash in the laboratory. Do not bring food or beverages into the laboratory.
• Wash your hands carefully before leaving the laboratory.
• Always wear safety glasses in the Biochemistry Laboratory!
• Read and follow instructions.
• Wear suitable clothing in the Biochemistry Laboratory. Sandals and shorts (unless covered by a lab coat) are not permitted in the lab.
• Wear latex gloves when working with dangerous biochemicals.

Project Report

The final project report should be in the general form of a research article, but will include an appendix with calculations. The following guidelines should be followed for preparation and submission of the final project report for the Biochemistry Module of the Advanced Chemistry Laboratory course.

• The final report should not exceed 15 pages excluding the title page and appendix of detailed sample calculations. Look at articles in journals such as Biochemistry for the general writing style for laboratory reports. More specific guidelines on report writing are provided in Section 4 of this document.

• All pages containing text (including the Calculations appendix) should be numbered. (Pagination on cover page may be excluded.)

• Text in laboratory reports should be font size 12. Figure legends and table headings may have smaller font sizes.
• Laboratory reports are due one week following "Day 6". The report must be given to the instructor. Do not put reports in the instructor's mailbox or under his/her door. DO NOT E-MAIL your reports!

• Delinquent laboratory reports will be graded according to the following schedule. Late reports that are not submitted directly to the instructor by the "late" deadline day will be given a grade of "0".

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<tr>
<th>Due Day</th>
<th>Late Report Accepted by 12 noon on the following...</th>
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<tr>
<td>Wednesday</td>
<td>Friday</td>
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<td>Friday</td>
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Lab reports must be printed clearly and legibly, and graphical data should be neatly presented. Follow standard usage regarding spelling, punctuation, and grammar. Sloppiness may result in point deduction.

Laboratory reports must include the following:

**Title Page** - Provide the experiment title, author, partner (if applicable), date(s) experiment performed, date due, date handed in, and the author's signature.

**Introduction** (15 points) - Present background for the experimental work described. State relevant concepts and hypotheses and the objectives of the experiment.

**Materials and Methods** (15 points) - Summarize the specialized reagents and their sources and equipment used in the experiment. Generally describe methods used especially where deviations to the protocol were made. Include the level of detail commonly found in published research articles and, only when necessary, add additional details.

**Results** (30 points) - Describe the data generated from the experiment. Tabulate or graph your raw data where possible.

**Discussion** (30 points) - State the overall conclusions from your experiment here. Discuss the significance, implications, comparisons of results to other work, etc. State and critically evaluate any assumptions that were made. Estimate the accuracy of your results. Discuss any observations that you found unusual or unexpected, and why they may have occurred. Note and discuss inconsistencies in your data that make drawing firm conclusions difficult. Discuss improvements that could be made in the laboratory hardware and apparatus that could improve your results.

**Calculations** (10 points) - Provide sample calculations made in the determination of the results generated. Note: Normally you will not find this section reported in the biochemical literature, but it is required here in order to evaluate your understanding of the work performed and independently evaluate the results. This section can be presented in neat, legible handwriting.

**References** - State any references made throughout the text of the research article. Glaring omission of references may result in point deduction.
Laboratory Notebook Maintenance

The recording and organization of a permanent record of laboratory observations is as important a technique to master as any of the experimental methods you learn. The research notebook is a day-by-day record of the progress of experimental work. It should reflect the integrity and honesty of the experimenter as well as the clarity of his or her thought. Therefore, the following are requirements for laboratory notebook maintenance for the Biochemistry module of this course.

- All experimental data, except instrument output, should be recorded in indelible ink in a bound laboratory notebook with pre-printed sequential page numbers.

- Appropriate lab notebooks are available at the bookstore. Loose-leaf or spiral-bound notebooks and loose pieces of paper are not acceptable.

- Students should sign the bottom of each page at the end of each laboratory period.

- Do not leave blank pages in a laboratory notebook.

- A lab notebook should include protocols, identification of samples, observations, and data.

- Record data and observations as you obtain or make them. Do not write on scraps of paper with the intention of transferring information to the lab notebook later.

- Do not worry if your notebook is a little messy.