

Chemistry 363 - Biochemistry Laboratory
Fall 2010 Syllabus

Instructor : Agnieszka (Agnes) Orlof

Teaching Assistant: Matthew Najor, Rui Wu

Location and Time of Sections: All students are required to enroll and participate in one laboratory section and one discussion section weekly. The choice of sections is shown below.

Laboratory sections: Tu 1:00-5:00 pm; We 1:30- 5:30 pm; F 8:30-12:30 pm; F 1:00-5:00 pm in FH-002

Discussion sections: W 8:15-9:05 am; Th 10:00-10:50 am in FH-129

Office hours: Outside of class, you may contact the Instructor during regularly scheduled Office Hours, W 9:20 – 1:00 pm. The office location, telephone number, and e-mail address are: Flanner Hall 002, (773) 508-2883 aorlof@luc.edu
If you are unable to contact the Instructor directly, or by voice or e-mail, you may leave a phone message with the Chemistry Departmental Office, (773) 508-3100.

Blackboard: This site contains current information for experiments and procedures.

Description and Objectives: This laboratory course is designed to simulate a research experience and to teach basic techniques utilized in a biochemistry laboratory. The course theme involves a comparative investigation of the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from various animal sources. All procedures required in lab will be found by the student in the library and proposed to the instructor(s) as a pre-lab exercise. Each two-student team will be working on GAPDH from either an aquatic or land animal source, e.g., turkey, tuna, pork, or chicken.

The objectives of the course are to:

1. learn and perform the techniques of protein isolation and purification;
2. characterize the protein based on size, shape, and stability; and
3. study enzyme kinetics.

The laboratory is an open-architecture environment. Student teams are expected to perform experiments during their normally scheduled laboratory session time; however, there will be opportunities to repeat certain procedures or experiments outside of the normally scheduled laboratory section period. Teams can work during normal business hours when the building is open, except when other laboratory sections are in session. The reason for this exception stems from our desire to have students who are scheduled for laboratory work in each particular section to enjoy complete and unfettered access to the limited resources and equipment that may be available. Student-teams who elect to pursue experiments outside of their normally scheduled laboratory section are responsible

for their experimental work and the appropriate use of all laboratory equipment and resources. Please do not request laboratory supervision from the instructor or TAs during non-laboratory sessions.

A weekly 50-minute discussion section will be used for the discussion of procedures, results, and conclusions. Students are expected to have completed their literature search for the next week's experiment prior to their designated discussion section. The discussion will be conducted as an open forum of questions and answers between students and the instructor. With the instructor's help, the students will compare the methods that they have found in the original literature and determine which methods are best suited for the lab. Upon the completion of the course, the students should draw conclusions and insights about the structure-function relationships of this enzyme.

Required Materials: All information used in the lab will be from the original literature found in the library. Although there will be no required laboratory text for this course, we expect you to acquire familiarity with procedures based upon your extensive knowledge of basic biochemistry that you learned during Chemistry 361 (review Ch. 3, pp. 65-78; Ch. 8, pp. 216-225; Ch. 16, pp. 441-443 of Stryer) and your use of library materials.

- Safety glasses: No student will be permitted to conduct research without eye protection;
- Lab coat is optional, but recommended; and
- Laboratory notebook with duplicate pages (this can be found at the bookstore)

Laboratory Experiments: Experiments 1-3 must be done in the prescribed order, but experiments 4-7 can be done in any order thereafter. All proposed experimental procedures will be discussed and approved by the lab instructor.

1. Check-in; buffer preparation; before week 2, conduct a literature search for GAPDH from the assigned source. To help you with task, the use of various resources within the library will be addressed in Chemistry 362. To locate specific published information on preparative procedures and other kinetic and molecular weight data for GAPDH from the source that you were assigned, you may find the following websites useful: www.expasy.org www.brenda-enzymes.info and www.ncbi.nih.gov .
2. Preparation and purification of GAPDH (allow 3 weeks)
3. Protein activity and concentration assays (allow 2 weeks)
4. Kinetics of GAPDH: determine K_m and V_{max} for substrates NAD^+ and G3P; this does not require fully purified material (allow 2 weeks)
5. Molecular weight determination: SDS-PAGE, gel filtration, and mass spectrometry; these experiments require approximately 4 mL of purified material with a concentration of at least 1 mg/mL (allow 2 weeks)

6. Protein stability: thermal denaturation; this does not require fully purified material (allow 2 weeks)
7. Comparison of results (allow 2 weeks)

Midterm and Final Papers: Each paper will be written in the format of a scientific journal: abstract, introduction, materials and methods, results, conclusion, and references. The midterm paper will incorporate information learned during the initial purification efforts, i.e., through experiment 3. The final paper will update the information from the midterm paper and describe the new experiments conducted since the fall break.

Grade Allocation:

15% Laboratory notebook. We expect you to follow a particular format for your research records, which is illustrated in the attached handout. Your notebook will be evaluated twice during the semester.

20% Laboratory performance. The TAs in consultation with the instructor will assess this score, which will be based on proper use of instrumentation, good laboratory and leadership skills and observation of safety techniques. You are expected to arrive to the laboratory on time and be prepared.

15% Discussion Section. The discussion score will be determined by the student's preparation and performance on quizzes. There are no make ups for quizzes.

20% Mid-Term paper. In addition to the ion-exchange chromatograph, this paper should include a protein activity table; masses and volumes of your protein sample during each step of the purification are needed to construct this table. Due date: 5:00 pm on Thursday, October 14, 2010.

30% Final paper. This paper will build on the midterm paper, and will compare kinetic and molecular weight data submitted by all teams. Students will be required to draw conclusions about GAPDH structure and function based upon an analysis of the collated data from all teams. Due date: 5:00 pm on Thursday, December 9, 2010.

If the midterm and/or final papers are submitted late, one-point deduction will be assessed for each day of tardiness.

Grading scale:

A = 100-88	A- = 87-83	B+ = 82-78
B = 77-73	B- = 72-68	C+ = 67-63
C = 62-58	C- = 57-53	D+ = 52-48
D = 47-40	F = Less than 40	