Chemistry 372 - Biochemistry Laboratory I
Fall 2016 Syllabus

Instructor: Agnes Orlof
Teaching Assistants: Jonathan Hill & Daniel Catlin

Laboratory sections: Tu or Th 8:30 AM- 12:30 Flanner Hall - Room 2 or 16
Discussion sections: Mon 10:25 – 11:15 am or 11:30 am- 12:20 pm Flanner Hall Room 7

Description and Objectives: This laboratory course is designed to simulate a research experience and to teach modern techniques utilized in a biochemistry laboratory, particularly the ones related to protein expression and purification. The course theme involves a comparative investigation of the enzyme ADP-glucose pyrophosphorylase from a bacterial source. Each two-or three-student team will be working on 2 forms of a recombinant ADP-glucose pyrophosphorylas.

The objectives of the course are to:

1. Learn and perform the techniques of a recombinant protein expression and purification
2. Characterize the protein that has been previously constructed with a tag for rapid purification.
3. Learn about how to improve on methodology published, literature search and presentation.

ADP-glucose pyrophosphorylase is an enzyme well studied, in which its purification has been described for a long time. Here in the course, we will try to improve it with modern techniques. There is a certain but moderate risk of facing challenges. The instructor and teaching assistants have a vast experience with the system to guide the students, but ultimately, we will experience the enthusiasm of research. That is finding new things. Some of the pedagogical goals are inspired by Kuhn, M.L., Figueroa, C.M., Aleanzi, M., Olsen, K.W., Iglesias, A.A. and Ballicora, M.A. (2010) "Bi-national and interdisciplinary course in enzyme engineering" *Biochem.Mol.Biol.Educ.* 38:370-379. [http://dx.doi.org/10.1002/bmb.20438]

> *…"that students work on real scientific problems during the laboratory sessions rather than performing a series of well-established experiments. While this may lead to unexpected difficulties, it is extremely advantageous for the student to learn how to approach a problem in an actual research environment"*

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 your TA during non-laboratory sessions.

A weekly 50-minute discussion section will be used for the discussion of procedures, results, and conclusions. 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:

- Safety glasses: No student will be permitted to conduct research without eye protection;

-Lab coat is optional, and
--Laboratory notebook

-Appropriate clothing must be worn that minimizes the potential chemical contact with your skin. No skin should be exposed on your feet or legs, so clothing that covers and protects your body from waist down should be worn.

Laboratory Experiments: All proposed experimental procedures will be discussed and approved by the lab instructor.

1. Check-in; solutions preparation ex. media, plasmid transformation, DNA purification, agarose gel electrophoresis (4 weeks)

2. Protein expression and purification of recombinant ADP-glucose pyrophosphorylase (allow 3 weeks)

3. Concentration assay, and SDS-PAGE (allow 2 weeks)

4. Kinetics of ADP-glucose pyrophosphorylase (allow 2-3 weeks). Screen for regulatory properties.

Lab reports: After completing a lab, each student will be required to turn in the lab report at the start of next lab.

Lab report should have the following sections:

I. **Title**
II. **Objective:** give a one-or-two sentence statement of the goals or purposes of the experiment
III. **Procedure:** describe what was actually done such as procedures, techniques, instrumentation and so on. It should be sufficiently detailed that the other experienced researchers would be able to repeat the work and obtain comparable results**.**

Hint: The procedure needs to be written in research paper style

IV. **Results/Calculations:** observations, equations, calculations, charts, figures, graphs etc which can be used effectively to present results clearly.
V. **Conclusion/Discussion:** the analysis and interpretation of your results. What do results mean? How do they relate to the objective of the experiment? Was the outcome successful? Outline the main conclusion of the project

A list of 5 lab reports (10 points each):

1) **Media preparation (Lab 1) and Plasmid Transformation (Lab 2)**
2) **DNA purification, digestion (Lab 3) and Agarose Gel Electrophoresis (Lab 4)**
3) **Protein expression (Lab 5) and Purification (Lab 6,7)**
4) **SDS-PAGE (Lab 8)**
5) **Malachite Green Assay (Lab 9,10,11)**

The lab reports must be typed. Results/Calculations are allowed to be hand-written.

If you miss a lab, the lab report will be due at the start of your next lab and half-credit will apply.

Final Paper: The paper will be written in the format of a scientific journal: abstract, introduction, materials and methods, results, conclusion, and references.

Grade Allocation:

50% Lab reports. We expect you to follow a particular format for your research records, which is illustrated in this syllabus.

10% Laboratory performance. The TA 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.

20% Discussion Section. The discussion score will be determined by the student's

participation, presentations and performance on quizzes. *There are no make ups for quizzes.*

20% Final paper. This paper will build on the lab reports, and will compare kinetic data submitted by other teams. Students will be required to draw conclusions about protein function based upon an analysis of the collated data from some other teams. Due date will be announced.

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

Class grades:

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 %	

Office hours: Outside of class, you may contact Agnes Orlof during regularly scheduled Office Hours, Mo 12:20-1:20 pm. The office location, telephone number, and e-mail address are: Flanner Hall 428, (773) 508-2883 aorlof@luc.edu

If you are unable to contact the Instructor directly, or e-mail, you may leave a phone message with the Chemistry Departmental Office, (773) 508-3100.

Sakai: This site contains current information for experiments and procedures and scores.

Academic integrity: We will follow the standards of the College of Arts and Sciences. In case a violation is detected, the particular assignment may receive a grade of zer