

- Instructor:**            Dr. Kevin P. Callahan – Office: ISHS A113;  
Phone: 385-8449; e-mail: kcallahan@sjfc.edu
- Office Hours:**        Thursday 12:00-2:00, or by arrangement.
- Textbook:**            Professor Callahan will provide relevant protocols and background information to students via blackboard.
- Lab Notebook:**      Permanently bound with numbered pages, capable of making removable duplicate copies.
- Goggles:**             Splash proof that meet the standards of all chemistry labs at SJFC

**COURSE OBJECTIVES:** Upon completion of this course, the student should be able to:

- Record information in a laboratory notebook
- Comply with laboratory safety
- Perform basic lab techniques including, protein expression, protein purification, SDS-PAGE gel analysis, DNA isolation and analysis, transformation, Enzymatic activity assays, UV/Vis spectrophotometry and protein structure viewing.
- Use problem-solving techniques and be creative in solving laboratory problems
- Compose formal laboratory reports involving critiquing and discussing laboratory outcomes

**PRE-LABS Assignments and LAB PARTICIPATION:** Prior to each laboratory session, you are required to complete the pre-lab assignments for each lab and outline the procedures you will be carrying out that day.

For pre-lab assignments: please write these on the first page of your lab notebook so a copy can be torn out of your lab book to turn into professor Callahan at the beginning of lab. Your procedure should go on the next full page of your lab notebook. It should include a clear outline, but need not repeat detailed procedures that you can refer to in your lab manual. Preparation and understanding of the lab will also be evaluated via unannounced and announced quizzes.

**Course Objectives/Overview:** This course is built around research and the skills and approaches necessary for research. The topic of the research is an enzyme, Malate Dehydrogenase, that plays a central role in metabolism. During this course you will establish a purification protocol for watermelon glyoxasomal malate dehydrogenase. You also learned how to perform kinetic assays of the enzyme and in the next several weeks will learn the basics of plasmid purification and the design of site directed mutants using the QuikChange protocol. This semester you will utilize some of these basic techniques in addition to more advanced techniques to put together a research project involving an investigation of structure-function relationships of glyoxasomal malate dehydrogenase. **The project will culminate in both a research presentation and a research paper that form the final exam in the course.**

**Overall Goals of Course –THINK, WRITE and TALK like a scientist.**

<b>GRADING</b>	Lab preparation and Lab performance	250 points
	Quizzes	100 points
	Home works	100 points
	Research Proposal and Final Paper	250 points

**Laboratory Reports:** Some general guidelines for all the lab reports can be found on blackboard under “lab report guidelines Callahan.docx” on.

### **Lab Notebooks**

When taking notes for the lab (lecture or self-made notes) or when doing protein structure work and bioinformatics searches in lab or for an assignment, these should each have their own entry in the lab book. **Lack of appropriate records in your lab notebook will negate any grade you received on your written reports.** If a record of an experiment and the data collected is not found in your notebook, there is no documentation that the experiment was conducted or that the data was actually collected. It is reasonable to expect that it will take you 20 minutes of each class to compile and organize your lab notebook for that day.

**I will spot check lab notebooks twice this semester. These dates will not be announced in advance. Your lab notebook should be organized and ready at all times.**

Your notebook will be graded based on the formatting (shown below), completeness, and the ability of another researcher (me) to exactly reproduce your experiment. NO grade will be assigned for neatness. However, if I can’t read the notebook then I can’t reproduce the experiment!

For each lab period, you must keep records that include:

- 1) Experiment title and date
  - a. This must be written on every page
- 2) Introduction/purpose/pre-lab questions
  - a. Include a 2-3 sentence statement of the objective/purpose of the lab exercises being performed.
  - b. Answer all assigned pre-lab questions
- 3) Materials and Procedure/protocol
  - a. You may either use the protocol provided to you or you may write out the procedure yourself. You must include in this section any deviations from the handout provided. This means that if you choose to print out the provided protocol, I expect to see clear handwritten notes where you made any modifications or needed to add additional information.
  - b. When you are repeating a procedure (such as a protein assay) you can skip the details and refer to the page in the notebook where the experiment was originally described. You must still record any deviations from the procedure you are referencing.
  - c. Where materials are. You will generate materials some weeks to be used in subsequent labs. Did you generate or isolate something that was stored in the -20 C freezer? The refrigerator? How was it labeled?

**The title, introduction/purpose/pre-lab questions and materials and methods sections should be completed before coming to lab.** (Deviations from the information presented in the laboratory handout will be recorded as you perform the laboratory experiments)

- 4) Data and results
  - a. All observations and data generated should be recorded at the time of the lab exercise. This will include copying or otherwise including tables, graphs, formulas, etc. This section should include all calculations, averages, and corrections to recorded data.
  - b. Graphs and tables must be labeled appropriately (axes, units, etc.). These may be prepared by a computer and taped into your notebook.
- 5) Discussion and conclusions
  - a. This section should include interpretations, conclusions, or suggestions regarding the data and results of the day's experiments. This must include a discussion of the expected results and why they were or were not obtained. Example questions to answer in this section are as follows:
    - i. What were the major points illustrated by the data?
    - ii. Do the results agree with the anticipated results?
    - iii. Does the data obtained make sense with your knowledge of biochemistry?
    - iv. Does the data indicate any potential follow-up experiments?
    - v. Do your results support or not support your hypothesis and why?

## LABORATORY SCHEDULE

### **Week 1**

Thurs. Sep 6 – Pre-lab assessment, Syllabus, Pipetting exercise, Transformation, Make LB media, **HW#1**  
Fri. Sep 5 – take picture of your plate – wrap and place in fridge

### **Week 2 – HW#1 is due.**

Wed. Sep 12 – inoculate starter 5mL culture  
Thurs. Sep 13 – Induce expression of MDH, paper discussion, and discuss proposal.  
Fri. Sep 14 – pellet cells and freeze

### **Week 3**

Thurs. Sep 20 – Lyse cells and bind to Nickel Resin. Bioinformatics, Structural analysis of MDH (MDH .ppt on BB).

### **Week 4- HW #2 due**

Thurs. Sep 27 – Elute protein, perform Bradford assay and SDS-PAGE  
Fri. Sep 28 – Destain gel and take picture

**After this lab you will be assigned a lab group at the University of San Diego that you will partner with.**

### **Week 5 –**

Thur. Oct 4 – WT activity assay – continuous and stop time. Design Primers for Mutagenesis

### **Week 6 - Proposals are due, oral presentation to collaborators in San Diego**

Thurs. Oct 11 – Enzyme kinetics with MDH

## Week 7 – Lab Quiz

Wed. Oct 17 – Inoculate DH5 $\alpha$  cells harboring mutant containing plasmid

Thurs. Oct 18 – Isolate plasmid, quantify DNA, transform plasmid into BL21-DE3 cells

## Week 8 -12 – Work on independent research projects

Oct 24

Oct 31

Nov 6

Nov 13

Nov 20

Nov 27

Dec 4

**Final Lab presentations describing Weeks 8,9,11,12 will be due Tuesday Dec 4.**

## BEHAVIOR IN THE LAB

**SAFETY IN THE LABORATORY:** Safety is extremely important in the BIOCHEMISTRY laboratory. The lab instructor will point out the fire extinguisher, showers, and eye wash in the lab. Be aware of their location, should you need them. All safety regulations must be observed during the lab period. The following is a set of safety rules that must be strictly observed in addition to the lab safety and regulations sheet requiring your signature:

- **SAFETY GOGGLES** are **REQUIRED AT ALL TIMES** during any experiment unless you are informed otherwise. **FAILURE TO COMPLY** with the goggle requirements may result in you being asked to **LEAVE THE LAB** and **RECEIVING A ZERO** on the report.

- avoid breathing chemical vapor or dust and avoid contact of chemicals with your skin
- do not start an experiment until you have carefully read and fully understand the laboratory procedure
- do not start an experiment over without permission<sup>[1]</sup><sub>[SEP]</sub>
- do not leave any experiments unattended<sup>[1]</sup><sub>[SEP]</sub>
- properly dispose of all chemicals when your work is finished

## **Disruptive behavior will NOT BE TOLERATED!**

Students are expected to behave as **adults** while in class. Be **RESPECTFUL** to everyone at all times and especially during classroom discussions. Continued disruptive behavior will not be tolerated and you will be asked to leave the classroom. Please be **ON TIME** to class. Please **TURN OFF** cell phones. **TEXTING, TWITTERING, WEB-SURFING or anything else you can do with your cell phone, iPod, iPad, etc.** will not be tolerated!

## **STATEMENT ON ACADEMIC INTEGRITY**

All students, regardless of level or school, are responsible for following the St. John Fisher College Academic Integrity Policy in addition to any other individual school's or program's academic expectations and/or professional standards. Every student is expected to demonstrate academic integrity in all academic

pursuits at all times. If a student suspects that another student has violated the Academic Integrity Policy, he or she should contact the instructor for that course and provide support for that suspicion. Any finding of responsibility and associated sanctions for a violation of the Academic Integrity Policy is retained per the College records policy.

**All students are expected to be familiar with the details of the Academic Honesty Policy (available via <http://catalog.sjfc.edu/undergraduate/2016-2017/academic-information/integrity.dot>).**

## **ACCOMMODATIONS FOR DISABILITIES**

In compliance with St. John Fisher College policy and applicable laws, appropriate academic accommodations are available to students with disabilities. All requests for accommodations must be supported by appropriate documentation/diagnosis and determined reasonable by St. John Fisher College. Students with documented disabilities (physical, learning, psychological) who may need academic accommodations are advised to refer to the Disability Services website (<http://home.sjfc.edu/AcademicAffairs/Disabilities/DisabilityOverview.asp>). Questions should be directed to the **Coordinator of Disability Services in the Office of Academic Affairs, Kearney 202**. Late notification will delay requested accommodations.

## **PROCESS TO RECONCILE ACADEMIC DECISIONS**

If a student wishes to appeal an academic decision of an instructor the process is outlined in the Undergraduate Catalog. The student should meet with the instructor to discuss the situation. If the student is not satisfied with the outcome and wishes to further pursue the matter the instructor should contact the instructor's supervisor. For Chemistry Faculty, the supervisor is the Chemistry Department Chair, Dr. Irene Kimaru.