

Syllabus for the Cell and Molecular Biology lab:

Class web site (where these notes and other information will be posted):
<http://carbon.cudenver.edu/~bstith/celllab.htm>

Skills we will be developing during this course:

1. Increasing our knowledge of major cellular concepts from General Cell Biology lecture
2. Specific topics include: protein folding and structure, making chemical solutions, lab calculations, lab safety (including MSDS reports on chemicals), cell culture, enzyme assay (map kinase), mitochondrial function, membrane electrophysiology, antibody-based assay (ELISA), cell signaling (action of hormones), microscopy, density gradient centrifugation, use of the spectrophotometer and pH meter, micropipetting, RT-PCR, bioinformatics, scientific units of measurement, and computer use in biology.
3. Reading and evaluating the original scientific literature
4. Maintaining a lab notebook and interpreting results through written lab reports (scientific writing, citing literature, organizing data and interpreting results)
5. Developing scientific graphs, understanding graphs from literature
6. Use of Word, PowerPoint and scientific graphics programs
7. Use of statistical analysis and statistics computer programs
8. Collecting data through use of sophisticated equipment and computer programs for controlling equipment and collecting data

Lab Notebook

Students will write own lab notebook; use an appropriate 3 ring notebook (check bookstore). The notebook will be graded at the middle (about March 15) and end of the class.

Use a table of contents in the front.

For each experimental procedure, list the steps in the techniques/methods (e.g., protein assay). View the discussion on what should be in lab notebooks on our course web site.

Tests:

There will be a test half way through the course (about March 15, covering material up to and including the map kinase (ERK) experiment. To study for the exam, review the lecture notes (PowerPoint presentations), pertinent text pages, and the experimental procedures. There will be a comprehensive final exam. There is no "lab practical."

Graduate Paper:

Graduate Students have a 15 page typed (single spaced, with illustrations) paper due week before finals. Chose a topic related to one of the labs and explore this topic in more depth. The format of the paper should be that noted below for the lab reports.

Lab Reports:

There will be 3 Lab reports: on Membrane physiology, Map kinase and the Mitochondrion. The lab reports should have the following sections: abstract, introduction

(literature background), results combined with discussion. Scientific references should be cited in the text and presented in a list (a bibliography at the end of the report). As the lab schedule varies, you will be notified as to the exact due date but typically, the due dates will be 2-3 weeks after the experiment is completed.

Topics (see course web site for detailed information and protocols):

1. Basic lab skills. Lab safety, glassware, use of pipettors, scales, making of buffers, use of PubMed, graphing with Sigmaplot, basic Student's t test statistics, Bradford protein assay, etc.
2. Protein Folding lab– use science computer lab. Use of ancillary text and Mage program to view components of protein structure. See my cell biology web site.
3. Cell culture lab; where students make buffers and culture the *Xenopus* oocyte
4. Simple Microscopy lab with oocytes. Use of a dissecting and monocular scope.
5. Cell signaling lab. After culturing the oocyte, add hormones to induce meiotic cell division. Insulin vs. progesterone action, ERK2/map kinase (using an ELISA), and the tyrosine kinase receptor pathway leads to the activation of cdc2 kinase.
6. Cell organelles; mitochondria. Isolate *Xenopus* liver mitochondria with Centrifugation and add substrates and use spectrophotometer to follow reactions. Effect of metformin on *Xenopus* mitochondria. What does the mitochondrion do? How does it do this?
7. Cell membrane lab; Use of “Neuro” computer program in science computer lab
8. Membrane potential lab; using iWorx probe and amplifiers connected to computers (recording and analysis software comes with amplifier), we will record the membrane potential of the plasma membrane and alter it through use of chemicals such as LPA (lysophosphatidic acid) and high K salt.
9. Fertilization; record Em, use ELISA to study process. Video and still digital photography to record events. Cell Division - mitosis in early embryo.
10. Computer Lab on molecular biology (bioinformatics); exercises on BLAST, Clustal analysis (held in science computer lab)
11. RT-PCR lab; ; isolation of mRNA, cDNA preparation and PCR.

Detailed Cell Biology Lab Policies (thanks to Dr. M. Greene):

Purpose of the laboratory:

The following laboratory exercises are designed to demonstrate important mechanisms and principles of cell biology and give you an opportunity to take a hands-on, experimental approach towards understanding these concepts. The concepts you will be learning in the laboratory section of this class will complement the materials learned in cell lecture and directly related to sections in the General Cell Biology textbook (*World of the Cell* by Becker et al.- also required for this lab course). The laboratory exercises will also help you learn about experimental methods along with the procedures involved in collection, documentation and analysis of scientific data. Further, assignments in the laboratory section will help you develop your scientific communication skills.

Attendance:

Attendance in lab is mandatory. Please keep in mind that the total of your laboratory score accounts for 33% of your total course grade. You are required to attend your assigned section. No make-up laboratory exams will be given under any circumstances; you will be responsible for documenting your absence to your instructor and reaching on an agreement for making-up the points. Often, you will receive the same score as your other laboratory exam. If you do not attend a laboratory, you will not be given points for any assignments turned in for that exercise unless you document your absence with your instructor immediately after returning to school. You will not be allowed to make-up a laboratory exercise under any circumstance.

Materials:

1. Lab exercises will available on the course web page. Students are required to download the week’s exercise and bring the hard copy to laboratory.
2. A laboratory notebook is required. You will record notes from each laboratory exercise. Bound laboratory data books or organized three-ring binders work well for this purpose. I will collect notebooks for a grade in the course and your performance on the laboratory practical exams will depend upon your ability to recall information about the experiments in an organized fashion. Keeping an organized lab notebook will help you in this endeavor.

All assignments must be turned in at the assigned time. If you miss an exam with a documented and approved excuse, a make-up assignment will be assigned by your lab instructor and Dr. Stith. Laboratory exercise question sheets will never be accepted late. Due to time constraints, poster presentations must be given on the assigned date.

Grading:

Lab Midterm	100 points
Notebook and Laboratory Exercises	50 points total
Three lab reports (30 pts each)	90 points total
<u>Lab Final Exam</u>	<u>100 points</u>

TOTAL **340 points**

Lab Groups:

You will work in laboratory groups consisting of 2-3 members for most laboratory exercises. Get to know your lab group members, as you will be working together for much of the semester. It would be helpful to exchange contact information among lab group members as well.

Clothing:

Use common sense when choosing what clothes to wear to laboratory. Do not wear your nicest outfits to lab section or, if you do, wear a lab coat.

Food and Drink:

Food and drink are not allowed in the laboratory, except when being used as part of a lab exercise. You will be using chemicals in the lab as well as expensive equipment, both of which in no way mix with drinking or eating at your lab station.

Equipment:

All of the equipment you need to conduct the laboratory exercises will be provided. We do ask that you treat the equipment with respect. For many laboratory exercises, computers will be at each lab station for data recording. Please avoid spilling, dropping or otherwise bothering the computers while conducting the exercise. iWorx computer-based physiological recording systems will be used for many of the exercise. Please follow the directions in this lab manual and those provided by your instructor to insure you are using the equipment safely and properly.

Waste Disposal:

There are separate containers in the lab for general waste, sharp objects, and biohazard materials. Please place all refuse in the proper container; these containers ensure the safe removal of waste from the laboratory. If you have a question, ask your instructor before you dump waste in the wrong container.

Cleanup:

You are responsible for cleaning your lab station and all equipment used in the exercise before you leave your laboratory section. The lab instructors will check your station before you leave the laboratory. Notify the laboratory instructor if there is a spill. Clean your glassware before leaving the lab so that you do not contaminate the next group's results.

Safety:

1. Follow instructions.
2. Please note the position of fire extinguishers, the safety shower, and the nearest exit.
3. Do not deviate from the laboratory manual instructions or those given to you by your instructor.
4. If you feel faint or ill during a laboratory exercise notify the members of your group and the lab instructor immediately. Do not exit the room without someone to attend to you.
5. Assume all chemical solutions used in the exercises are dangerous. Do not drink or eat any of the chemicals in lab, eat or drink in the laboratory, pipette by mouth, or carry reagents around the laboratory.

6. Wash your hands thoroughly before leaving the lab.
7. Close all reagent bottles after use.
8. If you spill, immediately notify your instructor so that it can be cleaned up safely.
9. Study the laboratory exercise before coming to lab to avoid confusion while conducting the experiment.
10. Keep your work station neat and well organized.
11. Never handle another person's blood without wearing gloves. Avoid spilling your own blood on the lab counter and always dispose of materials that have contacted blood in the biohazard container.