Biology 381: Biochemistry Laboratory

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Class: T or TH 1:00-5:00p (Hulings 206)

Office hours: W 2-3p; Th 10-11:30a; by appointment

TUES lab TA: Quinn Johnson, '19 Chemistry
THUR lab TA: Jessica Makori, '19 Biology

Course materials:

- An overview of each week's lab and relevant protocols will be available in print and electronic form prior to your scheduled lab day.

Course description: This lab will focus on the expression, purification, and characterization of proteins, with an emphasis on macromolecular interactions. The lab has three 'units': the first is focused on *in vitro* characterization of protein-small molecule interactions, the second will center on *in vivo* characterization of protein-protein interactions, and the third unit is a two-week independent project in which *you* decide what question to study (using the techniques you have learned in the first two units)! Attendance on your scheduled lab day is expected. Corequisite: BIOL 380.

LEARNING OBJECTIVES

After successfully completing this course, you should be able to:

- Conduct biochemical research experiments efficiently and accurately by paying careful attention to details as well as efficient time management practices
- Describe the basic biochemical principles underlying common modern biochemistry laboratory techniques, including protein overexpression, affinity purification, SDS-PAGE, binding assays
- Implement essential biochemistry laboratory skills such as accurately and reproducibly preparing reagents for experiments, designing and conducting properly controlled biochemical assays, and accurately processing and drawing conclusions from raw data
- Critically evaluate primary literature and data, and apply gathered information to new situations
- Effectively and constructively work together in a group situation to complete independent work
- Effectively communicate scientific concepts and/or experimental results to both expert and non-expert audiences using oral, visual, and written formats
- Increase problem solving skills such as: critical thinking, data analysis, and graphical analysis

ASSESSMENT OF LEARNING OBJECTIVES

Final grade distribution		Grading scale	
Lab notebook + questions	30%	A- to A	90-100%
Trp assay questions + protocol	10%	B- to B+	80-89%
Independent project proposal	10%	C- to C+	70-79%
Independent project presentation	30%	D	60-69%
Oral presentation	(15%)	F	< 60% D
Slides	(15%)		
Participation	20%		

This course is **NOT** graded on a curve to encourage you to work cooperatively with each other. If it becomes necessary to adjust the grading scale, I reserve the right to do so. Please remember that **you are not in competition with one another for grades**. Work with each other to learn the material!

Lab notebooks
The purpose of a lab notebook is for you to have a record of what you did in each lab period. Your notebooks are subject to review by me at any point in the semester, so make sure you are keeping your notebook up to date. You may use your own notebook as long as all the pages can be contained in one unit (i.e it passes the "shake test"—you can shake it and no pages fall out).

For each lab entry please include the following:

1. **Date and title** of the experiment.
2. **Purpose** of the experiment: include a hypothesis if applicable.
3. **Methods** used: simply cite the relevant portion of the lab manual (there is no need to rewrite the manual!) and more importantly, indicate any deviations from or alterations to the protocols.
4. **Results/data** obtained: observations, gel or western blot images, etc (for microscopy images, be sure to indicate the magnification, acquisition parameters, and where the file is stored).
5. **Conclusions**: What do your results tell you? Were there technical problems that made data interpretation difficult? Do you see a need for additional controls? Do you accept or reject your original hypothesis? What is the next step?

At the end of each lab you will have a few guiding questions to answer. The grade for your notebook also takes into account the effort and accuracy of your answers to these questions. I recommend completing these questions as soon as possible after the relevant lab period! The purpose of these questions is:

- Provide context for the experiments you will be conducting in lab and to help you analyze your data as you complete each experiment.
- The figures or content you create/write as part of these questions can either be directly used or slightly modified for your independent project proposal or independent project presentations (see below)!

Lab participation
Students are required to attend all labs! Please notify me in advance if you have a conflict due to a College-sponsored event or an interview. Since you will be working in groups, each group member should strive to complete their fair share of all lab activities. In addition, you may need to come into lab off-hours to complete experiments we were not able to finish during traditional lab hours. Your participation score will take into account whether you pulled your weight within the group, and whether your group did everything you could to complete your experiments. As with the classroom participation, half of your participation points will be assessed mid-way through the semester, while the remaining half will be assessed at the end of the semester. For the first 6 weeks of the term, you will be working in assigned groups of 2 or 3 in lab. For Weeks 7-9, you may choose to keep these partners for the independent project (see below) or switch to different groups depending on experimental interests.

Trp assay protocol
As part of the lab, you will be characterizing the activity of your protein using a ligand binding assay that utilizes tryptophan fluorescence to monitor changes in protein conformation. During Week 3, we will briefly go over the general principles of the assay, and I will assign a few papers for you to look at that use the assay. During Week 4, you will spend the first part of the lab period discussing your conclusions from the papers with your partner in order to determine the conditions and produce a protocol you will use during the second half of the lab. Your final protocol will be due at ***12pm the day before your scheduled lab day the following week (Week 5)***.

The purpose of this assignment is two-fold:

- Learning how to correctly execute the steps in a protocol you are given is an important first step towards applying what you've learned from class to a practical situation (this is true regardless of the subject—for example, remember the first time you followed a recipe and produced something you actually wanted to eat?). However, learning how to assemble a protocol "from scratch" using different sources is an equally valuable skill to have—it allows you to fully appreciate the logic behind each step (particularly when you have to decide if/where a step needs to occur) and also allows you to have some fun with regards to making changes/variations on something that is known (for example, adding chili pepper to spice up a dish that normally doesn't have that).
- On a more general note, we are swimming in a vast sea of information that will continue expanding at an exponential rate. Learning how to efficiently sift through this information to identify relevant/accurate content and then to synthesize this content into something that is actually useful to you (and potentially others) is an **incredibly** important skill. Assembling a protocol that you will use is a narrow application of this process to our class, but it builds skills that will be helpful to you regardless of your field.

Independent project proposal
Using the techniques you learned in the first half of the term, you and your lab partners will design an independent project of your own design to be carried out during Weeks 7-8. You may keep your original lab partners or you may find new ones if experimental interests differ between groups.

- During Week 4, you will have some down time during the lab period that you can use to being brainstorming project ideas (with your partner(s), classmates, and/or myself!).
- During Week 5 your group will need to schedule a 15 minute meeting with me outside of class to discuss your independent project ideas. You should come to this meeting *prepared* with your idea for your project, a rough timeline what you plan to do in the two weeks, as well as a list of reagents you require for your project.
- Your group's **project proposal** is due ***Sunday October 21st*** and should include a strong rationale for your project (citing primary literature and/or data from the first half of the semester), the methods you will use (including requests for microscope time, which can be scheduled on the Google calendar), special materials you would like me to order, and any additional requests.

I will provide a list of protein constructs and resources that are readily available to you, and will make every effort to secure additional resources you may require to carry out your experiments!

******* If you start thinking about your project earlier in the term and decide you would like to do your project with protein constructs other than the ones I have (for example, specific mutations you would like to test in the receptor): **Let me know as soon as possible (preferably by Week 5 at the latest)** then I can do my best to generate those constructs for your project *******

Independent project presentations
Together, you and your lab partner(s) will present the results of your independent investigation during a shared lab period with BIOL 281 in Week 9. Plan for a 7-10min presentation, followed by 3-5min of questions from the audience. The grade for your presentation is broken down as follows (more detailed rubrics/guidelines will be provided separately):

Oral presentation	15%
Visuals (slides, etc)	15%

- Being able to clearly, concisely, and accurately communicate information about your work to an audience is an **incredibly** valuable skill. It is also something that takes a lot of practice (I still get nervous standing before you all on the first day of class!). The oral presentation portion of the grade takes into account the clarity and quality of the verbal presentation, while the visual portion of the grade takes into account the quality of the visuals used as part of your presentation.

ADDITIONAL POLICIES

(the policies enumerated in the BIOL 380 syllabus apply here as well)

Group work
Learning how to work well together in a group is a critical skill that will serve you all well regardless of the career path you choose to pursue. However, it is equally critical that **each group member contributes** to the work at hand, and I reserve the right to inquire about how the work was divided between individual group members. If you feel an arrangement has become imbalanced, please come see me privately so we can work out an arrangement to suit everyone.

Course feedback and input
One of my goals as your instructor is to create an inclusive, non-threatening environment for our class, such that you feel comfortable and welcome as a member of our classroom community to share your thoughts/questions with me and/or your peers. In the past I have received valuable feedback from students regarding things they think could be improved; however, this feedback often comes at the end of the term, limiting the benefits of the feedback to only future classes and not the current class. Thus in an effort to try to improve our class in real-time, I have created a gmail account (carletonfeedback@gmail.com; password: carl3t0n!) to serve as a digital "suggestion box" for our class. If you see something in class that you think could be adjusted to improve our classroom environment, you can log-in to this account and use it to send me an anonymous email with your thoughts.

Please use this account ***conscientiously*** (for example, don't use this email to sign-up for online promos or spam) and ***constructively***. I will do my best to address concerns as they arise!

COURSE SCHEDULE

Lab resources for each week will be posted on Moodle. The syllabus will be updated to reflect any changes. The tentative schedule is below, but is subject to change.

Week	Date	Research question	Lab topic(s)	Experiments	Major Assignments due
1	Sept 11-13	*In vitro* Biochemistry	Intro to AMPA receptors/LBD Protein expression in *E. coli*: general overview	Make buffers Introduction to project	
2	Sept 18-20	*In vitro* Biochemistry: protein purification	Overview of Ni-affinity chromatography	Ni-NTA chromatography Prepare SDS-PAGE samples Dialyze protein**	
3	Sept 25-27	*In vitro* Biochemistry: protein characterization	Methods for protein characterization: SDS-PAGE Methods for protein quantification	SDS-PAGE: Run the gel, stain, & image Bradford assay on dialyzed protein	
4	Oct 2-4	*In vitro* Biochemistry: small molecule ligand interactions	Ligand binding assays: tryptophan fluorescence assay	Design & write up protocol for Trp fluorescence assay Trp fluorescence assay + data workup*	Trp fluorescence protocol Due: 12pm your lab day the following week
5	Oct 9-11	*In vivo* Biochemistry: protein-protein interactions	Protein-protein interactions: co-immunoprecipitation	In vivo: BCA assay Set up Co-IP* Brainstorm independent project ideas	Independent project meetings
6	Oct 16-18	*In vivo* Biochemistry: protein-protein interactions	Methods for protein characterization: Western Blots	SDS-PAGE Western Blot: Transfer, block, blot*	Independent project proposal Due: 10/21 5pm
7	Oct 23-25	You decide! Independent projects	---	Independent projects*	
8	Oct 30-Nov 1	You decide! Independent projects	---	Independent projects*	
9	Nov 6-8	---	Shared lab with BIOL 281!	Independent presentations	Independent project presentation
10	Nov 13-15	**NO LAB!**	**CONGRATS!**	**YOU DID IT!**	**WOO!**

*May require coming in outside of class (dependent on experimental conditions)
**I will help take care of certain steps for you outside of class these days