Biochemistry Laboratory Molecular Techniques 2
BB 494/594 (3 credits)
Winter 2016
OSU Corvallis Campus

Laboratory sections	MW 14:00-16:50, TuTh 9:00-11:50, TuTh 13:00-15:50, ALS 0023
Lecture	Tu 16:00-16:50, LINC 303
Professor	Dr. Ryan Mehl, ryan.mehl@oregonstate.edu
Instructor	Dr. Kari van Zee, 737-1773, vanzeek@science.oregonstate.edu
Office Hours:	To be announced
Teaching Assistants	Rachel Henson, Kelsey Kean, Ramya Raman
Prerequisites	BB 493/593 Biochemistry Laboratory Molecular Techniques 1, BI 315, BB 315 or Instructor permission. It is expected that students have mastered the simple organic chemistry of functional groups, general chemistry problem solving in stoichiometry, pH and equilibrium calculations and simple algebraic skills such as equation solving and use of logarithms.
Required Materials	*Lab protocols and readings* ***w***ill be available through the BB494/594 course portal. Students are expected to print out protocols and bring with them to lab or bring laptops/tablets so they can access materials during lab. Two computers are available in the teaching lab.

Students should also bring a lab notebook, PC-formatted USB drive to each lab session, safety glasses, and lab coat to each session (lab coats and safety glasses are also available to check out for the term from the teaching lab). Students' laptops will be needed in lab to perform literature searches and analyze molecular structures.

Course Description

This advanced-level laboratory focuses on protein biochemistry. Proteins play vital roles in most biological processes as catalysts for physiological reactions, as regulators for those reactions, or as structural framework around which these processes can occur. Students will work in small interdisciplinary teams to design, execute, evaluate, and communicate guided-research projects focusing on protein structure and function.

Learning Outcomes:

After completing this course students will be able to:

1. Define specialized language of the biochemistry laboratory.

2. Describe concepts fundamental to biochemistry techniques, including concepts relating to protein purification, spectrophotometry, assay design, and characterization of proteins.
3. Operate safely biochemistry laboratory equipment, including micropipets, thermocyclers, centrifuges, gel electrophoresis chambers, power supplies, incubators, and autoclaves.
4. Demonstrate quantitative skills by preparing accurately and reproducibly reagents and solutions for experiments.
5. Design experiments, including the proper controls, to express, purify, and characterize recombinant proteins.
6. Collect and analyze experimental data.
7. Evaluate their experimental findings in the context of scientific literature and communicate an understanding of core concepts, experimental details, and calculations used in each experiment in written tests, manuscripts, and oral presentations.

Additional Learning Outcomes for BB 594 graduate students

Graduate students gain additional experience in critical analysis and problems solving skills through a literature-based term proposal project and after completing the course will be able to

1. Evaluate scientific contributions of recent publications in protein biochemistry.
2. Defend their analysis in written and oral communications.
3. Propose next-step approaches to advancing the area of research.

Lectures

Lectures are held at 4:00 pm on Tuesdays in LINC 3003. Students registered for the course are expected to attend all lectures and are responsible for mastering the material presented in the lectures and in any assigned reading.

Winter 2016 BB 494 Weekly Schedule will be provided as a separate document.

What the Professors Expect of the Students

- Student will arrive on time and come prepared for lab classes by studying the handouts or posted readings prior to class.
- Student will actively keep notes in a lab notebook before, during, and after the performance of each experiment.
- Student will follow safety rules of the laboratory and wear appropriate personal protective equipment and clothing in the lab.
- Student will be able to adapt a general protocol to accomplish specific tasks as necessary. The protocols used are similar to those used in research labs.
- Student will read the experimental write-up before lectures and lab and come with questions regarding the experiments.
- If there is difficulty in understanding the concepts or problems, the student is expected to obtain help from the teaching assistants or the professor. Letting problems go until the last minute will not provide for a successful classroom experience.
- Students may need to invest time out-side of the scheduled course hours to complete experiments. Instructors will work to accommodate schedule constraints.

Grading

BB 494

Laboratory and Lecture Deliverables	15%
Laboratory Notebooks	20%
Scientific manuscript draft 1	10%
Scientific manuscript draft 2	10%
Peer reviews of draft 2	10%
Final Paper	25%
Group Research Presentation	10%

BB 594

Laboratory Notebooks	20%
Scientific manuscript draft 1	10%
Scientific manuscript draft 2	10%
Peer reviews of draft 2	10%
Final Paper	25%
Group Research Presentation	10%
Graduate term project	15%

All students are expected to meet the lecture, laboratory, and project deliverables described on the 2016 Weekly Schedule and clarified below. Lab and lecture deliverables are worth a total of 15% of the final grade (or 1% each deliverable). Project deliverables include drafts of the scientific manuscript, peer review and group research presentation.

Laboratory Deliverables: Weekly lab deliverables are outlined on the 2016 BB 494 Schedule. Each student is expected to turn in a lab deliverable handout according to the schedule to receive credit for meeting the deliverable. In addition, students are expected to be punctual and prepared for class, and to conduct their experiments safely, efficiently and with consideration for others on their team and in the lab. Students are expected to plan effectively and complete all experimental work during assigned lab periods. Students are also expected to follow lab rules, demonstrate care in carrying out protocols, keep track of samples, store them appropriately with clear labels, be responsible for the fate of plates, proteins, etc., ensure proper disposal of waste and maintain a clean and tidy workspace.

Lecture Deliverables: Students will complete weekly literature assignments and be expected to participate in discussions. The first five weeks there will be a common paper for all student sections to read. At the end of each of the first four lectures you will turn in a typed short summary, a single question you would want to ask on this paper and the proper citation of this paper. We will discuss the paper at the end of weekly lectures and in lab periods. Weeks 6-9 there will lab group literature cross-fertilization assignments. Each group will select a paper related to their research direction that they find interesting and/useful. Each group member will write a summary and question at the end of their notebook with the citation of the paper. In the first 30 minutes of lab we will discuss the papers. TAs will look for these literature assignments when they grade the notebooks.

Laboratory Notebooks:

Notebooks will be collected on at the end of Week 10 and will be graded by the TAs during finals week. Keeping and maintaining an accurate and complete laboratory notebook is an essential part of experimental science. Notebooks should contain:

- Purpose, prior to lab
- Dated experiments throughout the notebook
- Pre-lab and planning write-up, in the notebook; prior to lab.
- Calculations clearly shown
- Notes made during the experiment
- Clearly presented and well-labeled results in the form of photos, figures, tables
- Analysis and conclusions clearly stated
- Quality of the results: The professors consider the ability to successfully execute, rather than just understand, experiments a key component of experimental science.

Remember, function trumps form. We are not looking for beautiful notebooks, but will be looking for well-organized notebooks that allow relevant information to be retrieved easily. Neatness naturally helps in this endeavor.

Scientific Manuscript:

The goal of the research project will be to write a Biochemistry style manuscript discussing the structure of your proteins, what you learned and what you want to tell the world. This manuscript will be built in stages moving toward the final draft. Details for what should be covered in each draft will be provided on the course website/portal.

Draft one: This will focus primarily on two sections: (1) the introduction of your protein, hypothesis of your study, and what you hope to learn about your protein and (2) the methods containing the bulk of the experimental section. Results may also be included if available. Due date is listed on the weekly schedule.

Draft two: This will include all the information and corrections from draft one and add the results, figures, captions and discussion sections of the manuscript. Due date is listed on the weekly schedule.

Peer Review: Peer review of scientific manuscripts and grants is standard for all scientists. This functions to maintain quality and clarity of all reviewed scientific work. This process also helps the reviewer learn how to present information clearly. Each student will be given manuscripts from two classmates to review and provide feedback. You will be given the manuscripts at the beginning of week 8 and turn them back in at the end of the week. You will be evaluated on how well you review the manuscript.

Final Draft: Due as hard copy on **Friday of Week 10 by 4 pm**. This will be the final polished ACS Biochemistry style manuscript including all feedback and scientific results.

Group Research Presentation:
At the end of the semester you and your group should be experts on your enzyme. The last lab periods will be dedicated to group presentations approximately 40 minutes in length. Groups will present their team's work and lead a class discussion about how it compares to the known literature on that enzyme.

Safety: **Protective eye wear and lab coats are required at all times when working with chemicals and equipment in the lab.** *The safe handling and proper disposal of toxic chemicals, biological waste, and broken glass in a biochemistry laboratory is required. Professors and TAs will answer questions regarding the location and methods of disposal. Safety or disposal violations will also lead to significant loss of points.*

Absences: If, for some unavoidable reason, you need to be absent for a class period, *it is your responsibility to speak to or email the instructor ahead of time and arrange with your lab partners to make up the work that you miss*.

University Policies

Statement Regarding Students with Disabilities
Accommodations are collaborative efforts between students, faculty and Disability Access Services (DAS). Students with accommodations approved through DAS are responsible for contacting the faculty member in charge of the course prior to or during the first week of the term to discuss accommodations. Students who believe they are eligible for accommodations but who have not yet obtained approval through DAS should contact DAS immediately at 541-737-4098.

Student Conduct

The Department of Biochemistry/Biophysics and the Biology Program follow the university policies on student conduct. These can be found at http://studentlife.oregonstate.edu/studentconduct/offenses-0

Cheating or plagiarism by students is subject to the disciplinary process outlined in the Student Conduct Regulations. Students are expected to be honest and ethical in their academic work. Academic dishonesty is defined as an intentional act of deception in one of the following areas:

* cheating- use or attempted use of unauthorized materials, information or study aids
* fabrication- falsification or invention of any information
* assisting- helping another commit an act of academic dishonesty
* tampering- altering or interfering with evaluation instruments and documents
* plagiarism- representing the words or ideas of another person as one's own

Behaviors disruptive to the learning environment will not be tolerated and will be referred to the Office of Student Conduct for disciplinary action.

"The goal of Oregon State University is to provide students with the knowledge, skill and wisdom they need to contribute to society. Our rules are formulated to guarantee each student's freedom to learn and to protect the fundamental rights of others. People must treat each other with dignity and respect in order for scholarship to thrive. Behaviors that are disruptive to teaching and learning will not be tolerated, and will be referred to the Student Conduct Program for disciplinary action. Behaviors that create a hostile, offensive or intimidating environment based on gender, race, ethnicity, color, religion, age, disability, marital status or sexual orientation will be referred to the Affirmative Action Office.

BB-594 Term Project

Description: This project will be a mini research proposal. The idea is to extend what we have been doing with our paper summaries into what would be the next generation work. Below I have provided you with a paper and you will repeat the summary process on it as you have done before (writing a single paragraph to answer the three key points).

Scientific Paper to be announced at the beginning of the term.

In addition to this paragraph you will add the next research step surrounding this area of your interest. This next generation work can take the area in whatever direction you are excited about or think is easiest. It can be a structural biology direction, a signaling direction, enzymology, drug design, application in medicine or material science, or simply an extension of protein characterization to clarify the proposed model or interactions. You are welcome to explore other options as well, just discuss them with us first. Your research proposal should be broken into three defined sections: Problem Significance, Approach, Expected Outcomes.

(1) **Problem Significance**: What is the gap in knowledge and why is it significant (or important to fill)?
(2) **Approach**: What are the steps, tools, methods, or approach you would use to fill this gap and type of information would they provide?
(3) **Expected Outcomes**: What are your expected outcomes or new knowledge? If you have trouble with expected outcomes it helps to include potential problems one expects to encounter.

We have seen many methods to solve problems in the papers we have read. You can draw from these approaches and are welcome to read as much additional literature you would like to craft your proposal. To assist you, additional papers are included in the folder as resources to put the protein in the context of the field and a unique method or tool that we have not seen before. Neither of these papers need including in your report but are meant to help provide a broader scope. You are welcome to brainstorm with each other and with me as much as you would like.

Grading Rubric: The project will be worth 100 points and will be 15% of your total course grade.

The standard summaries will be graded out of a total of **25 points**.
4 points: following the technical instructions completely.
21 points: For an effective first paragraph summarizing the paper. This should touch on the gap of knowledge in the field that is being filled with this publication, any unique approaches needed for the advance, major contributions and their meaning.
The remaining 75 points will be distributed equally for each of the three section of your proposal. Attention should be paid to the logic and clarity of your ideas, arguments and proposed solutions. Meaning a proposal that is really novel and a significant advance only has merit if the problem, approach and outcomes are logical.

Deadlines and Details:

1. Your individually written report must be in your own words.
2. The written summary **must** be single-spaced and typed in 12 point Times, Times New Roman, or in 11 point Arial font.
3. The summary **must** be no longer than 2 page in length including figure and captions if needed. The references can be on a third page. It must contain your name and date in the header.
4. You are expected to use clear and coherent writing with correct use of grammar, punctuation etc.
5. The written report is due by *Friday* of Week 10 by 4:30 pm. Late papers will lose one letter grade per day.
6. Both a paper copy and an electronic copy must be submitted. Electronic copies must be submitted to Ryan Mehl (ryan.mehl@oregonstate.edu). Paper copies may be submitted to the BB receptionist, in the BB office.

BB494/594—Research Rotation with Dr. Mehl/Dr. van Zee Winter 2016

Prior to first session of lab: Each student will be assigned and notified by email which of the two proteins they work with during the term. Students are expected to complete background reading and brief assignment through Canvas prior to first lab session. Team assignments will be provided in lab. Please note the various lecture, lab, and project deliverables.

Week 1	**1/4-8**	**Design Group Research Project**
Lab Deliverable:		By start Day 1, complete and bring printed Canvas assignment on "Introduction to Your Enzyme"**.**
Lecture Deliverable:		Literature Assignment Handout for Week 1

Lecture 1 (T): (LINC 303, 4-4:50 pm): Studying Protein structure/function—Pymol Visualization of example protein and introduction to expanded translation. Course overview.

Lab Day 1: <u>Laptops needed</u>
Check in, discussion of assigned protein, team assignments
Literature search, download Pymol, research protein platforms and potential NCAAs, study crystal structures. Identify mutations available for student projects. Read selection of papers posted on Canvas.
Lab Day 2:
Finalize selection of team's research protein and mutants. Begin to develop hypothesis and select NCAAs and sites to be studied throughout term. Organize expression media components, glassware, and containers.

Week 2	**1/11-15**	**Protein Expression and SDS-PAGE analysis**
Lecture Deliverable:		Bring hypothesis to lecture and turn in Literature Assignment Handout for Week 2
Lab Deliverable:		By end of lab Wed. or Thurs. each person submits handout with selected sites and ncAAs to evaluate during term; identify plasmid strains/cell lines needed.

Lecture 2: (LINC 303, 4-4:50 pm): Expanded Translation Machinery/Protein Expression Media
Peer discussions of hypothesis-each team should bring a hypothesis to lecture to share and discuss.

Lab Day 1:
Prepare autoinduction media, start expression of wild-type protein—culture for 30-40 hours
Prepare SDS-PAGE solutions beginning of week.
Define hypothesis, select sites and unnatural amino acids to incorporate.
Lab Day 2:
Harvest cells—measure OD, spin down pellets
Analyze wild-type protein production by SDS-PAGE end of week (crude gel analysis).
Prep protein purification buffers

Week 3 **1/18-22** **Protein Purification and Initial Characterization**

Lecture Deliverable: Turn in Literature Assignment Handout for Week 3
Lab Deliverable: None

Lecture 3: (LINC 303, 4-4:50 pm) Protein Purification

Monday Holiday= Martin Luther King Day (no lab) Monday section will start expressions Wednesday afternoon and harvest Friday morning/afternoon. At least one team member needs to be available Friday

Lab:
Purify wild-type protein from cells.
Start expression of both wild-type and mutants with NCAAs.
Identify assay. Understand choice of assay.
Make assay/storage buffers.
Harvest cells—measure OD, spin down pellets (crude gel analysis)
Desalt protein into assay/storage buffer.
Analysis of purified proteins by SDS-PAGE.
Determine if wild-type protein is active.

Week 4 **1/25-29 Protein Characterization – Structure/Function Relationship**

Lecture Deliverable: Turn in Literature Assignment Handout for Week 4
Lab Deliverable: By end of lab Wed or Thurs, submit typed and printed experimental procedure for expression and purification of wild-type enzyme. Submit a figure with appropriate labeling and legend of gels showing crude extracts and pure protein.
Lecture 4: Kinetic Assays—developing a kinetic assay

Lab:
Determine protein concentration.
Determine if wild-type pure protein is active.
Purify wild-type and mutants.
Desalt proteins into assay/storage buffer.
Analysis of purified proteins by SDS-PAGE.
Determine if wild-type protein is active.

Week 5 **2/1-5**

Project Deliverable: Draft 1 Research Paper due Monday 9 am, hard copy, turn in to ALS 2141—Kari's office.
Lecture Deliverable: Turn in Literature Assignment Handout for Week 5
Lab Deliverable: By end of lab Wed or Thurs, submit typed and printed experimental procedure for qualitative and quantitative assay. Submit a figure with

appropriate labeling and legend of qualitative assay on wild-type protein.

Lecture 5: Dr. Victor Hsu—Introduction to BB496 project (tentative)

Lab:
Obtain catalytic constants wild-type and NCAA-containing enzymes with kinetic assays, modifying as necessary.

Week 6 **2/8-12**

Lecture Deliverable: Literature assignment now team-based and recorded in lab notebook.

Lab Deliverable: By end of lab Wed or Thurs, submit typed and printed experimental methods for purification and quantification of wild-type and mutant proteins. Submit a figure with appropriate labeling and legend of gels showing crude extracts and purified wild-type and ncAA mutant proteins.

Lab:
Obtain catalytic constants wild-type and NCAA-containing enzymes with kinetic assays, modifying as necessary
Design and execute additional study on pure proteins including testing of wild type and NCAA-containing enzymes with kinetic assays.

Week 7 ***2/15-2/19***

Project Deliverable: Draft 2 of Research Paper due Thursday at 4 pm in BB teaching lab, 3 printed copies (black and white is fine) with Draft 1+ comments attached to one of the copies.

Lecture Deliverable: Literature assignment now team-based and recorded in lab notebook
Lab Deliverable: None

Lab:
Design and execute studies on pure protein including testing of wild type and NCAA-containing enzymes with kinetic assays.
MS of purified protein samples (depending on MS availability).

Week 8 **2/22-2/26**

Project Deliverable: Peer evaluation of 2 assigned papers completed by Thursday, 2/25 at 4 pm. Turn in to BBTL ALS 0023. Draft 2 peer evaluation assignment handed out Monday/Tuesday in lab.

Lecture Deliverable: Literature assignment now team-based and recorded in lab notebook.

Lab Deliverable: By end of lab Wed or Thurs, submit typed and printed experimental method for quantitative enzymatic assay. Submit a figure with appropriate labeling and legend of kinetic evaluation of wild-type and if possible ncAA mutant proteins.

Lab:
Design and execute studies on pure protein including testing of wild type and NCAA-containing enzymes with kinetic assays.
Design/execute individual assays for protein structure and function.

Week 9 ***2/29-3/4***

Lecture Deliverable: Literature assignment now team-based and recorded in lab notebook.

Lab Deliverable: By end of lab Wed or Thurs, submit typed and printed experimental methods of unique protein assay. Submit a figure with appropriate labeling and legend of Lineweaver Burk plot of wild-type and ncAA mutant proteins and a table with kinetic constants for all three proteins with error bars.

Lab:
Design/execute individual assays for protein structure and function.
Wrap up all lab work. Clean-up by end of lab on Wed or Thursday (no exceptions).
Create presentations.

Week 10 **3/7-3/11**

Project Deliverables:
Mon/Tues Presentations during lab sections
Wed/Thurs Presentations during lab sections

Lecture Deliverable: None.
Lab Deliverable: None

Final Draft (color printed version) and Lab notebooks due by Friday 3/11 at 4 pm to Kari's office ALS 2141

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