CHM4910 — Undergraduate Research Experience in Biochemistry — Spring 2019

Credits: 3; Prereq: CHM2045 or the equivalent.

This course is a 'CURE' course (Course-based Undergraduate Research Experience). It is designed around several different active research projects in biochemistry in the labs of several faculty in the Department of Chemistry at UF.

Instructors	Sections	Coordinates
Angerhofer	31A7	Tel.: 392 9489 (office, CLB318A), 392 0541 (office, LEI214A), email: alex@chem.ufl.edu. O.H.: W-11 period in CLB318A, and by appointment.
Bruner	3E97	Tel.: 392-0525 (office, JHH302E) email: bruner@chem.ufl.edu. O.H.: T: 9am – 10am, R: 2pm – 3pm in JHH302E.
Fanucci	35DC	Tel.: 392-2345 (office, LEI 311E) email: fanucci@chem.ufl.edu O.H.: T 10:30am – 11:30am
Harris	35DB	Tel.: 392-9865 (office, JHH302F) email: harris@chem.ufl.edu O.H.: M-4 period in JHH302F and by appointment.

TAs	Katie Dunleavy, k.dunleavy@chem.ufl.edu, #35DC, O.H.: tba Loc Tien Huynh, lochuynh@ufl.edu, #35DB, O.H.: tba Gengnan Li, gengnanli@chem.ufl.edu, #3E97, O.H.: W: 2pm – 3pm. Tony Pastore, atpastore09@chem.ufl.edu, #31A7, O.H.: tba	
Sections	#35DB/3E97, M6-10 Bruner/Li & Harris/Huynh	#31A7/35DC, T6-10 Angerhofer/Pastore & Fanucci/Dunleavy

Class Meeting Times	W-3 period, 9:35am–10:25am in Joseph Hernandez Hall 221 (lectures) M6-10 or T6-10, depending on section, in LEI 200 (lab work)
Holidays	01/21 (MLK Day), 03/04 – 08 (Spring Break), 03/25–26 (Reading Days, no classes).
Class Text	There is no textbook assigned for this course. Reading material will be assigned on a weekly basis with material accessible through canvas.
Pre-Lab Homework	Pre-Lab homework will be assigned and is due the next lab day at beginning of lab periods.
Points Earnable	Pre-lab homework @ 20% of total grade. Laboratory Notebook @ 10% of total grade. Research related assignments @ 25% each of total grade. Lab reports @ 25% of total grade. Oral lab report @ 20% of total grade. Total earnable points are 100%.
Grading Scheme[1]	A: ≥ 90.0% 90.0% > A− ≥ 86.0% 86.0% > B+ ≥ 83.0% 83.0% > B ≥ 80.0% 80.0% > B− ≥ 77.0% 77.0% > C+ ≥ 73.0% 73.0% > C ≥ 69.0% 69.0% > D+ ≥ 66.0% 66.0% > D ≥ 63.0% 63.0% > D− ≥ 60.0% 60.0% > E.

1 see **https://catalog.ufl.edu/ugrad/current/regulations/info/grades.aspx** for more info on UF grade policies.

Course Schedule (tentative):

Date	Week	Topic	Instructor
01/07–08/19	1	No labs	
01/09/19	1	Introduction and Lab Safety	Angerhofer
01/14–15/19	2	Lab work: pipetting techniques and mass measurements	
01/16/19	2	Review of fundamentals of biology (proteins, nucleic acids, translation, ...)	Bruner
01/21–22/19	3	No Labs (MLK Day)	
01/23/19	3	Review of library/database resources.	Librarian
01/28–29/19	4	Bradford Assay on BSA	
01/30/19	4	PCR/Cloning Strategies I	Harris
02/04–05/19	5	Primer Design	
02/06/19	5	PCR/Cloning Strategies II	Harris
02/11–12/19	6	PCR amplification and gel electrophoresis	
02/13/19	6	PCR/Cloning Strategies III	Harris
02/18–19/19	7	DNA purification, quantitation, and transformation	
02/20/19	7	PyMOL for visualization of protein structures	Bruner
02/25–26/19	8	DNA purification, quantitation, and transformation, cont'd	
02/27/19	8	Introduction to specific proteins I	Bruner/ Harris
03/11–12/19	9	Pilot Expression of recombinant proteins	
03/13/19	9	Introduction to specific proteins II	Angerhofer /Fanucci
03/18–19/19	10	Large scale expression of recombinant proteins, SDS-PAGE Analysis	
03/20/19	10	Protein purification	Fanucci
03/25–26/19	11	Protein purification and concentration, Bradford assay	
03/27/19	11	Enzyme Assays	Fanucci
04/01–02/19	12	Enzyme Assays	
04/03/19	12	Biophysical Methods for Enzyme Characterization I	Angerhofer
04/08–09/19	13	Enzyme Experiments (CD, EPR, etc.)	
04/10/19	13	Biophysical Methods for Enzyme Characterization II	Bruner
04/15–16/19	14	Enzyme Experiments cont'd	
04/17/19	14	Biophysics/Biochemistry Research in Context	Fanucci
04/22–23/19	15	Student Presentations	
04/24/19	15	Review of Activities throughout the semester and outlook	Angerhofer

Further Important Information:

1. **Overview and Goals:** As a CURE class this course is designed to lead the student into cutting edge research as it is practiced in the labs of the instructors. Students will learn the fundamentals on how research in Biochemistry and Biophysics is performed. They will develop their own hypotheses and test them by making new site-directed mutants of specific enzymes. The focus of the lab activities lies on acquisition of skills, trouble shooting, problem solving, and reproducibility. Grading emphasizes process skills not research outcomes. Approximately 20% of the time is spend on repeating potentially significant experiments where students are challenged to "repeat their critical results."
2. **Course Description:** The course is an advanced laboratory course that is built around a full semester project supporting current research activities in the labs of Drs. Angerhofer, Bruner, Fanucci, and Harris. The project will require a focus on techniques for the preparation and quantitative analysis of proteins and other macromolecules, presenting students with a broad spectrum of techniques, approaches, and concepts of contemporary biochemistry in the context of their application to research. You will learn aspects of DNA purification and analysis, protein expression and quantification, enzyme purification, enzymatic characterization, chromatography, electrophoresis, immunological techniques, and spectroscopic analysis. You will design your own experimental procedures to address a research question that you will develop, continually analyze,

evaluate, and report on. You will do all of this while demonstrating safe laboratory practices and keeping a complete and organized notebook.

Students will work in small groups of up to 6 students on a specific protein under the direction of a principal investigator. The laboratory component of the course will take place either M or T periods 6 through 10. There will also be a common lecture component for all students in the course on W-3 period. Site directed mutagenesis (SDM) is the alteration of a protein at a specific position of its amino acid chain to change its behavior. Such an intervention may lead to changes in a protein's activity for enzymatic catalysis, its stability, and its interaction with other biomolecules such as RNA, DNA, or other proteins. The technology has been developed several decades ago is mature and provides a rational approach to protein engineering and design for various applications such as: investigation of structure-function relationships of important biological proteins, improving catalytic efficiency of enzymes, the study of the mechanisms of genetically inherited diseases, *etc*. In the Spring 2019 term the following proteins will be under investigation in this course, oxalate decarboxylase (OxDC), yeast proteinase A inhibitor 3 (IA_3), microviridins (serine protease inhibitors), and the bacterial enzyme ribonuclease P (RNase P). The faculty guiding these projects are: A. Angerhofer (OxDC), S. Bruner (microviridins), G. Fanucci (IA_3), and M. Harris (RNase P). A brief description of each project follows:

3. **Project Descriptions:**

Oxalate Decarboxylase catalyzes the redox-neutral disproportionation reaction of mono-protonated oxalate into carbon dioxide and formate. Oxalic acid, the conjugate acid of oxalate, is the most common naturally occuring toxin in our food. It is produced by plants and oxalate overload is the leading cause of kidney stones in humans and animals. Oxalate scaling is also a problem in various industrial processes where plant material has to be processed. The enzymatic mechanism of OxDC is not well understood owing to the complexity of two separate Mn-centers in the subunit of the protein and the fact that the enzyme is able to carry out two very different chemistries, *i.e.*, decarboxylase and oxidase of oxalate. SDM will be applied to probe the function of specific amino acid residues near the active site of the enzyme with the goal to rationally develop mutant enzymes with enhanced stability and activity in the neutral pH range for (A) a better understanding of the catalytic mechanism at the molecular level, and (B) potential future applications in the medical intervention for kidney stones and prevention of oxalate scaling in industrial process streams.

Yeast Proteinase A Inhibitor 3: The yeast proteinase A (YPRA) inhibitor, IA_3 of *Saccharomyces cerevisiae* consists of 68 amino acid residues and forms an α-helix when bound to YPRA. IA_3 is classified as an intrinsically disordered protein as it remains unstructured in the absence of YPRA. The α-helix of IA_3 resides in the N-terminal region of the protein, leaving the C-terminus structurally unresolved and disordered. To probe the understanding of the helical folding of IA_3 and to investigate the changes to overall helical intensity, mutational analysis in the N-terminus of IA_3 will be performed. Site-directed mutagenesis, protein overexpression, protein growth, and protein purification will be completed in this lab to then study the structural changes to IA_3 due to residue mutation using circular dichroism spectroscopy.

Microviridins: Peptide natural products are an important class with a wide range of bioactivities, such as antimicrobial, antiviral, and anticancer. The biosyntheses of these drugs share a common paradigm starting with the ribosomal production of a precursor peptide and subsequent processing by enzymes that chemically modify the peptide, generating a final, bioactive product. Microviridins are a family of peptide natural products featuring a unique cage-like architecture. This class binds serine proteases, key therapeutic targets in numerous biological processes (*e.g.*, infection, inflammation, and apoptosis) and are proven targets of FDA-approved drugs. This project will examine the structure and mechanism of the biosynthetic enzymes through mutagenesis, biochemical assays and protein structure determination. The overall goal is to rationally engineer microviridins that specifically bind a therapeutically relevant serine protease as a drug lead.

RNase P: Antibiotic resistance is a major health problem worldwide. The bacterial enzyme ribonuclease P is an attractive drug target because it is essential for cell growth, however the structure of the enzyme varies between humans and bacteria allowing for the development of specific inhibitors. RNase P is a ribonucleoprotein enzyme composed of a large RNA molecule and a smaller protein subunit. We are in the early stages of cloning and expressing the RNA and protein subunits of RNase P from over thirty different pathogenic bacteria in order to determine their structure, mechanism and for use in high throughput screening.

4. **Learning Outcomes:**

(1) Identify, locate and use the primary literature.

(2) Develop a testable and falsifiable hypothesis based upon review of related primary literature approaches, and design appropriate experiments and controls to test your hypothesis.

(3) Design, construct and validate one or more mutants to interrogate your hypothesis.

(4) Use various biochemical and biophysical approaches to characterize, compare and contrast, mutant and wild type proteins.

(5) Calculate kinetic parameters of an enzyme from experimental data and use kinetic parameters to compare wild type and mutant enzymes.

(6) Explain the importance of and keep an accurate laboratory notebook.

(7) Communicate scientific results in the form of written lab reports and a final powerpoint presentation. Use visual and verbal tools to explain concepts and data.
(8) Work with peers to evaluate data, apply knowledge to data and interpret data. Give and take directions to be an effective team member.

5. **Class Meeting Times:** The class meets in JHH221 W-3 period. Class discussion will start on time. Please be there a couple of minutes early. There are two lab meeting times for the different sections, M6-10 and T6-10 periods in LEI 200 (see page 1 of this syllabus for details). These lab periods will be used for the various experimental activities in this course. Note that some activities may take place in the PIs' labs. Please also note that there may be lab activities necessary outside these specified class meeting times and you are expected to make reasonable arrangements with the PI and his/her graduate students to get your work done. Make sure to pay attention to relevant announcements.
6. **Teamwork:** You will be assigned to a team for the semester. As part of this team, you will develop a hypothesis, design and complete experiments to test the group's hypothesis. As part of your team work, you will evaluate your team and your team will evaluate you. Your group work reflects the real-world experience of scientists,that is team–based studies and interdisciplinary cohorts. From your group work, you will gain experience working with peers to evaluate, interpret, and debate data/ethical issues pertaining to the course materials.
7. **Pre-Lab Homework:** Homework assignments, Pre-Lab reading, and associated quizzes will be turned in throughout the semester and must be completed on time to be adequately prepared for the next lab period. See the detailed grade breakdown above and refer to the schedule of activity dates provided. Late homework and assignments will not be accepted for a grade.
8. **Laboratory Notebook:** Your laboratory notebook should be an accurate record of what you do in the lab, and should contain notes and calculations as well as appropriate comments to the lab you are working on. You should enter the lab with your notebook prepared for the day's experiments. A major function of a lab notebook is to allow another competent scientist to reproduce exactly your experiment.
9. **Group Specific Projects:** The instructors, Drs. Angerhofer, Bruner, Fanucci, and Harris all work on different proteins/enzymes. Students will be assigned in groups to these different projects. While there are many commonalities between them they are distinct in their approach and research goals. You will be primarily responsible to pursue the goals of the group you are assigned to. However, you should also pay attention to discussions and presentations of other groups in order to gain an appreciation for the breadth of biochemistry and biophysics research.
10. **Lab Reports:** Each student will be responsible for written lab reports. These reports roughly parallel the progression of activities throughout the semester. Taken together they should reflect the draft of a research grade publication and therefore reflect the components typically found in the peer-reviewed papers. The following lab reports will be required and will be announced at least one week before their due dates:
    (1) Hypothesis: Needs to be based on a literature overview of your project and include a discussion on how you will be testing the hypothesis.
    (2) Experimental Procedures: Will need to include a description of the protocols and procedures used in designing your mutant protein, its preparation and purification.
    (3) Experimental Results: Will need to document visually and in writing the results obtained in your experimental lab work, including assay results.
    (4) Discussion: The last lab report will deal with a discussion of the results, conclusions drawn from your work and potential outlook for future research work on this project.
11. **Oral Reports:** Each student will give an oral report during the last week of the semester. These will take place during the time blocks reserved for the labs on April 22 and 23 and will be done by section. Each student has 15 minutes for their presentation followed by 2 to 3 minutes of discussion. Rooms for the presentation will be announced.
12. **Canvas:** Access your Canvas e-learning account by clicking on the 'Log-In to E-Learning' link on the web site, http://lss.at.ufl.edu/ where you will have to supply your Gatorlink credentials to log in. Please, do this at your earliest convenience and make yourself familiar. Canvas will be primarily used by TAs and the instructor to communicate with the class. Please make sure to monitor the announcements on a regular basis. There may occasionally be assignments on Canvas that need to be completed before class. If you experience technical problems when using Canvas, please contact the UFIT helpdesk (http://helpdesk.ufl.edu/, 352-392-4357 M-F from 8:00am till 5:00pm, email helpdesk@ufl.edu, or go to: http://helpdesk.ufl.edu/e-learning-support/).
13. **Class Attendance:** Regular attendance is essential for your success in this class. However, we will not do roll-calls. Repeated absence in class and labs will make it very difficult to succeed in this research course. For further information on UF's attendance policies which are in effect for this course, see: https://catalog.ufl.edu/ugrad/current/regulations/info/attendance.aspx .
14. **Study Habits:** The course demands on average 10 – 12 hours/week of work outside of class. It is expected that you read the assigned reading materials before coming to class/lab. The instructor will build on this material and you are expected to be able to follow in-class discussion and in-lab activities. The course demands a regular sustained effort throughout the semester. The experiments

build successively on each other and you have to succeed in an earlier experiment to be able to work on later ones.

15. **Office Hours:** The instructors and graduate student TAs offer several office hours spread over the whole week. The detailed times and locations are listed on the first page of this syllabus. This is time we set aside for you. Take advantage of it. Please note that the instructor and all TAs are available to help students in any of the CURE class sections. You are not limited to only the TA assigned to your section. However, your assigned TA will most likely be the most familiar with the protein you are working with.
16. **Online Course Evaluation:** Students are expected to provide feedback on the quality of instruction in this course by completing online evaluations at https://evaluations.ufl.edu. Evaluations are typically open during the last two or three weeks of the semester. Announcements will be made to students about the specific times when they are open. Summary results of these assessments are available to students at https://evaluations.ufl.edu/results/.
17. **Students with Disabilities:** Students requiring special accommodations should register with the Dean of Students Office (http://www.dso.ufl.edu/, 352-392-1261) and the Disability Resource Center (DRC, https://www.dso.ufl.edu/drc, 352-392-8565, email: accessUF@dso.ufl.edu), and present documentation from that office to the instructor.
18. **Counseling Services:** The University of Florida provides counseling services for students, staff, and faculty. See http://www.counseling.ufl.edu/cwc/. If you or a friend are in distress, call (352) 392-1575 (available 24/7), email umatter@ufl.edu, or walk in for an emergency consultation during regular service hours (8:00am – 5:00pm) at the Radio Road Site, 3190 Radio Rd., or the Peabody Hall Site, on the 4th floor of Peabody Hall, adjacent to Criser Hall. For other hours or weekends, call the Alachua County Crisis Center, (352) 264-6789. For sexual assault recovery services call the Student Health Care Center at (352) 392-1161. For life-threatening emergencies always call 911.
19. **Emergency Numbers and Web Sites:**
    - UFPD (UF Police Department): In case of emergency dial 911. The UF campus police non-emergency number is (352) 392-1111. Their web site: http://www.police.ufl.edu/,
    - UF Emergency management: (352) 273-2100. https://emergency.ufl.edu/,
    - Infirmary (student health center): (352) 392-1161, http://shcc.ufl.edu/.
    - EH&S (Environmental Health & Safety): (352) 392-1591, http://www.ehs.ufl.edu/.
20. **Other Academic Resources:** UF provides several other resources for students, such as
    - Library Support can be obtained here: http://cms.uflib.ufl.edu/ask, where you can find various ways to receive assistance with respect to using the libraries or finding resources.
    - The Career Resource Center is located on level One in the Reitz Union, (352) 392-1601, and provides career assistance and counseling. Refer to http://www.crc.ufl.edu/ for further info.
    - The Teaching Center is located in Broward Hall, main phone (352) 392-2010 or appointment phone (352) 392-6420, and provides students with tutoring services and counseling regarding general study skills. Refer to http://teachingcenter.ufl.edu/ for further info. It may also provide employment opportunities as tutors for well qualified students.
    - The Writing Studio is located at 302, Tigert Hall, (352) 846-1138, and provides help with brainstorming, formatting, and writing papers, see: https://writing.ufl.edu/writing-studio/.
    - The Ombuds Office is located at 31 Tigert Hall, (352) 392-1308, and provides students assistance in resolving problems and conflicts that arise in the course of interacting with the University of Florida. By considering problems in an unbiased way, the Ombuds works to achieve a fair resolution and works to protect the rights of all parties involved. For further information go to http://www.ombuds.ufl.edu/ or refer to the official complaints policy here: https://www.dso.ufl.edu/documents/UF_Complaints_policy.pdf.
21. **Cell Phone Etiquette:** Please put all cell phones or other electronic devices on "**silent mode**" during all class and lab periods. Please do not leave the classroom during lecture to make a phone call. Thank you!
22. **Honor Code:** This class will operate under the policies of the student honor code which can be found at: https://www.dso.ufl.edu/sccr/process/student-conduct-honor-code/. The students, instructor, and TAs are honor-bound to comply with the Honors Pledge: **We, the members of the University of Florida community, pledge to hold ourselves and our peers to the highest standards of honesty and integrity.** You are expected to exhibit behavior consistent with this commitment to the UF academic community, and on all work submitted for credit at the University of Florida, the following pledge is either required or implied: *"On my honor, I have neither given nor received unauthorized aid in doing this assignment*." It is assumed that you will complete all work independently in each course unless the instructor provides explicit permission for you to collaborate on course tasks. Furthermore, as part of your obligation to uphold the Honor Code, you should report any condition that facilitates academic misconduct to appropriate personnel. It is your individual responsibility to know and comply with all university policies and procedures regarding academic integrity and the Student Honor Code. Violations of the Honor Code at the University of Florida will not be tolerated. Violations will be reported to the Dean of Students Office for consideration of disciplinary action. For more information regarding the Student Honor Code,

please see: https://www.dso.ufl.edu/sccr/process/student-conduct-honor-code/.

23. **Disclaimer:** This syllabus represents our current plans and objectives. If those need to change as the semester progresses, then the changes will be communicated to the class clearly.

If you have further questions, please contact us. Have a great semester!
Sincerely,

Alexander Angerhofer Steven Bruner Gail Fanucci Mike Harris