CHM4910 — Undergraduate Research Experience in Biochemistry — Spring 2023

Credits: 3; Prerequisite: CHM2045 or the equivalent.

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

Faculty	Section	Faculty Contact and O.H.	Teaching assistant
Butcher	U101	Tel. 846-3392 (office, SFH302B) email: butcher@chem.ufl.edu O.H.: TBA in SFH302B	Weijie Xu email: xuweijie@chem.ufl.edu
Bruner	U102	Tel.: 392-0525 (office, SFH302E) email: bruner@chem.ufl.edu O.H.: TBA in SFH302E	Thisuri Wanniarachchi email: twanniarachchi@chem.ufl.edu
Rudolf	U103	Tel.: 294-7221 (office, SFH302G) email: jrudolf@chem.ufl.edu O.H.: TBA in SFH302G	Tyler Alsup email: tyleralsup@chem.ufl.edu
Fanucci	U104	Tel.: 392-2345 (office, CLB311F) email: fanucci@chem.ufl.edu O.H.: TBA in LEI311F	Tina Li email: tianyan.li@chem.ufl.edu
Angerhofer	U105	Tel.: 392-9489 (office, CLB318A) email: alex@chem.ufl.edu O.H.: W-4 (10:40-11:30am) CLB318A and by appointment	Zain Becerra email: zbecerra@ufl.edu
Eddy	U106	Tel.: 294-1048 (office, SFH302C) email: matthew.eddy@chem.ufl.edu O.H.: TBA in SFH302C	Kara Anazia email: karaanazia@chem.ufl.edu
Harris	U107	Tel: 352-932-1742 (office, SFH302F) email: harris@chem.ufl.edu O.H.: TBA in SFH302F	Kandice Simmons email: simmonsk@chem.ufl.edu

Class Meeting Times:

Lectures: Wed., period 3 (9:35–10:25am) in SFH221

Lab time: Mon./Wed. period 6-10 (U101, U102, U103) or Tues./Thurs. 6-10 (U104, U105, U106, U107) in each faculty member's research labs

Holidays: 1/16 (MLK Day), 3/11 – 3/18 (Spring Break), 4/27–4/28 (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.

Grading:

Quizzes = 10%

Laboratory Notebook including pre-lab assignments = 15%

Research-related assignments = 25%

Lab reports = 25%
Oral lab report = 25%

Grading Scheme:

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

Course Schedule (tentative):

Date	Week	Topic	Faculty
1/11	1	Introduction	Bruner
1/9-1/13	1	No labs	
1/18	2	Fundamentals of biology	Bruner
1/16-1/20	2	Mon./Tues.: Intro to pipetting, mass measurements, safety Wed./Thurs.: Library/database resources and discussion of individual projects	
1/25	3	PCR/Cloning strategies I	Bruner
1/23-1/27	3	Only Wed./Thurs. labs (MLK day): Primer design, PCR/cloning practice	
2/1	4	PCR/cloning strategies II	Bruner
1/30-2/3	4	PCR/cloning on individual projects	
2/8	5	PCR/cloning strategies III	Bruner
2/6-2/10	5	PCR amplification and gel electrophoresis	
2/15	6	PyMOL for visualization of protein structures	Bruner
2/13-2/17	6	DNA purification, quantitation, and transformation	
2/22	7	Introduction to specific proteins I	Rudolf/Fanucci
2/20-2/24	7	Pilot expression of recombinant proteins	
3/1	8	Introduction to specific proteins II	Butcher/Bruner
2/27-3/3	8	Small scale expression/screening conditions	
3/8	9	Introduction to specific proteins III	Angerhofer/Eddy
3/6-3/10	9	Large scale expression of recombinant proteins	
3/22	10	Introduction to specific proteins IV	Harris
3/20-3/24	10	Protein purification and concentration, Bradford assay	
3/29	11	Protein purification/Western blotting	Bruner

3/27-3/31	11	SDS-PAGE analysis, native PAGE	
4/5	12	Biophysical methods for enzyme characterization I	Matt Eddy
4/3-4/7	12	Functional assays	
4/12	13	Biophysical methods for enzyme characterization II	Matt Eddy
4/10-4/14	13	Enzyme experiments (CD, EPR, etc.)	
4/19	14	Biophysical methods for enzyme characterization II	Bruner
4/17-4/21	14	Enzyme Experiments cont'd	
5/1-5/2	15	Mon. and Tues : Student presentations	

Further Important Information:

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."

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 the participating faculty. 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 4 students on a specific protein under the direction of a principal investigator. Site directed mutagenesis 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.

Project Descriptions:

Acyl-CoA oxidases (Butcher): Nematodes are small roundworms that are very diverse (over 25,000 characterized species) and can be either free-living, such as the model

organism *Caenorhabditis elegans*, or parasitic, causing widespread disease in plants and animals (including humans). Despite the huge impact of nematodes on agriculture and human health, surprisingly little is known about chemical signaling in members of this important animal phylum. *C. elegans* produces a complex mixture of ascaroside pheromones in order to communicate with other nematodes and to coordinate its development and behavior. Acyl-CoA oxidase (ACOX) enzymes, which participate in beta-oxidation cycles that shorten the side chains of the ascarosides, regulate the mixture of pheromones produced. We have shown that different ACOX enzymes have different side chain length preferences and thus act as gatekeepers for the production of specific pheromones, thereby controlling the chemical message that is produced by nematodes. Using X-ray crystallography and molecular modeling, we have provided a molecular basis for the substrate specificities of the ACOX enzymes and have uncovered why some of these enzymes have a very broad substrate range while others are quite specific. Surprisingly, we have shown that the ACOX enzymes also bind to ATP. Thus, pheromone production may be coupled to the metabolic state of the worm. In this course, we will utilize site-directed mutagenesis, biochemical assays, and protein structure determination to investigate how ATP regulates the activity of the ACOX enzymes.

Mycosporines (Bruner): Mycosporine-like amino acids (MAAs) are a group of UV absorbing, photostable small molecule secondary metabolites biosynthesized by marine algae to provide protection from exposure to high solar radiation. The chemical structure of MAAs consists of a functionalized cyclohexenone or cyclohexenimine core having absorption maxima around 310-360 nm. The inherent high extinction coefficients makes this class of natural products attractive as natural sunscreens. This project will examine the structure and mechanism of MAA biosynthetic enzymes through protein structure determination, mutagenesis, and biochemical assays. Overall, we aim to expand the chemistry of MAA biosynthetic enzymes through protein engineering to design and development of a range of structurally diverse novel small molecules for broad-spectrum novel sunscreen formulations.

Terpene synthases (Rudolf): Terpene synthases (Rudolf): Terpenoids, or terpenes, are the largest and most structurally diverse family of natural products. Terpene synthases (TSs) are a family of enzymes that begin most terpenoid biosynthetic pathways. TSs utilize carbocation chemistry to transform acyclic precursors into structurally and stereochemically complex skeletons. Their exquisite abilities to control the conformation of the acyclic substrate, stabilize the numerous reactive carbocations, and selectively quench the carbocation are breathtaking, but not fully understood. Sequence analysis of each TS cannot predict what product(s) is formed and even a single point mutation, which disrupts carbocation stability, can completely alter product formation and/or distribution. Therefore, the characterization of bacterial TSs will advance the fields of natural products, terpene enzymology, and terpene utilization. The functional, mechanistic, and structural characterization of these enzymes will also build a foundation of knowledge that will guide future genome mining efforts while ultimately leading to the ability to make rational structure predictions based solely on genomic information. In this project, selected TSs will be tested for terpene synthase activity. TSs of interest may be engineered into variants that possess amino acid mutations in and around the active site, revealing the functional roles of active site amino acids.

Lymphoid enhancement factor or T-cell factor (Fanucci): Lymphoid enhancement factor or T-cell factor (Lef/Tcf) transcription factors are intrinsically disordered proteins in charge of stem cell regeneration as part of the Wnt signaling cascade. This class of transcription factors bind

to β-catenin in the nucleus of the cell, downstream to the Wnt signal initiation located extracellularly to this complex. The trafficking of β-catenin into the cell is a process, that when perturbed can result in improper activation of the Lef/Tcf transcription factors resulting in cancerous stem cell renewal. The four different Lef/Tcf transcription factors differ in their adopted structure when bound to β-catenin, even though the amino acid sequence is rather conserved. The Lef/Tcf -β-catenin binding interaction will be studied to uncover these differences in structure, related to their activation or repression of certain Wnt targeted genes. Site-directed mutagenesis, protein overexpression, protein growth, and protein purification will be completed in this lab to then study the functional ability of these transcription factors due to residue mutation using binding assays.

Oxalate Decarboxylase (Angerhofer): Oxalic acid, the conjugate acid of oxalate, is the most common naturally occurring toxin in our food. It is produced by plants and oxalate overload is the leading cause of kidney stones in humans and animals. The bacterial and fungal bicupin enzyme OxDC catalyzes the redox-neutral disproportionation reaction of mono-protonated oxalate into carbon dioxide and formate. The reaction mechanism involves a long-range electron transfer between two redox-active Mn ions across protein boundaries in the hexameric quaternary structure of OxDC which may also be important for its secondary and tertiary chemistry, *i.e.*, oxalate oxidase and catalase. In soil bacteria the enzyme is stress-induced by low pH. It is only active at pH levels below 6 and shows its maximum efficiency at or below pH4. In order for OxDC to be useful in healthcare settings (*e.g.*, as a sensor for oxalate concentration in blood or urine) or in industrial environments (*e.g.*, to help avoid oxalate crusts in feeder pipes in the paper and pulp industry), the enzyme would need to be trained to be active at normal pH7. In this CURE project the students will utilize directed evolution technology to make and isolate OxDC isozymes where the maximum of activity is shifted to higher pH. The molecular mechanism by which the pH dependence of activity is controlled will also be investigated.

G-Protein Coupled Receptors (Eddy): Our perception of our surroundings—through taste, smell, and sight—arises from a family of sensory proteins on the surfaces of all human cells called G protein-coupled receptors (GPCRs). Proteins in the same family recognize many clinical and street drugs, including opioids and cannabinoids, as well as hormones and metabolites. These extraordinary sensory proteins are involved in nearly every physiological process and are targeted by over one third of FDA-approved drugs to treat many diseases. We are investigating how GPCRs interact with other proteins inside human cells to form signaling complexes that ultimately generate physiological responses. To study the interactions of GPCRs with other signaling proteins, we are preparing variants (i.e., mutant proteins) for both GPCRs and their partner proteins to investigate the role of specific amino acid substitutions on forming signaling complexes. The information we learn from these experiments will provide important data on the first steps of drug recognition and signaling in human cells.

A second project area in the Eddy lab is the production and investigation of protein-polymer bioconjugates. Proteins are important therapeutics that have wide ranging applications from cancer treatments to mitigating inflammation. However, unlike small molecules protein therapeutics are less stable and more likely to be recognized by the human body as a foreign entity and destroyed. Chemically conjugating a polymer such as polyethylene glycol (PEG) to proteins can significantly improve their clinical benefits, resulting in over 30 FDA-approved protein-polymer drugs. However little experimental data are available to guide the design of

such bioconjugate therapeutics. In this project, students will help design and study new protein-polymer conjugates in order to identify criteria that could be used to produce new bioconjugates with enhanced therapeutic potential.

Ribonucleotide reductase (Harris): Cancer continues to be is a major devastating disease that affects millions in the US every year. The human enzyme ribonucleotide reductase (RNR) converts ribonucleotides to deoxyribonucleotide for DNA synthesis and is therefore essential for cell division. Human RNR is a current chemotherapeutic drug target for late stage and difficult to treat cancers because of its important role in DNA replication. The allosteric regulation of RNR is complex and involves two nucleotide binding sites that regulate activity and substrate specificity, respectively. To pinpoint key amino acid residues involved in allosteric signaling we performed molecular dynamics simulations and phylogenetic comparative sequence analysis. The candidate amino acids we identified are likely to be involved in the protein conformational changes that link the allosteric nucleotide binding sites. To test this hypothesis and set the stage for more detailed structural and biophysical studies of RNR regulation, we are creating a series of mutant RNR proteins with specific amino acid substitutions at positions identified as involved in protein dynamics. The information gained studying these mutant enzymes will not only reveal fundamentally important principles of protein allostery, but provide design principles for the development of new chemotherapeutic agents that target RNR.

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.

Class Meeting Times: The class meets in SFH221 W-3 period. Class discussion will start on time. Please be there a couple of minutes early. There are different lab meeting times and locations for the different sections, MW6-10 and TR6-10 periods in the research labs of the different groups (see page 1 of this syllabus for details). These lab periods will be used for the various experimental activities in this course. Not all weeks will be as busy and utilize both afternoons of lab time fully. However, there may be weeks where your lab activities make it necessary to spend extra time outside these specified class meeting times and you are expected to make reasonable arrangements with the faculty member and his/her graduate students to get your work done. Make sure to pay attention to relevant announcements.

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.

Pre-Lab Work: It is important to show up for lab prepared. There will be assignments that need to be completed before you start your lab work. This could be reading assignments, pre-lab quizzes, or other activities that may need to be performed on canvas or in your lab notebook. The TAs will check your pre-lab work and grade it. Late pre-lab assignments cannot be accepted for a grade.

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.

Post-Lecture Quizzes: In order to maximize your learning in the lecture part of the course, online quizzes will be given 3-4 times per semester, each covering 3-4 lecture's worth of material.

Section-Specific Research-Related Projects: The faculty teaching this course 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.

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. The due date will be in late January/early February and will be announced in class and on canvas at least a week ahead of time.

(2) Experimental Procedures: Will need to include a description of the protocols and procedures used in designing your mutant protein, its preparation and purification. This lab report will be due by the end of February or in early March. The timeline depends on the research progress of the teams. The deadline for this lab report will be announced at least a week ahead of time.

(3) Experimental Results: Will need to document visually and in writing the results obtained in your experimental lab work, including assay results. This lab report will be due

toward the end of the semester, in mid-April when most of the results have been acquired and need to be documented.

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 18 and 19 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.

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/).

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.

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.

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.

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/.

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.

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.

Emergency numbers and Websites:

- 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/

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.

Cellphone 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!

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/.

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.

Steve Bruner, Rebecca Butcher, Jeffrey Rudolf, Gail Fanucci, Alexander Angerhofer, Michael Harris and Matt Eddy