CHM4910 — Undergraduate Research Experience in Biochemistry — Spring 2024

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.

Course Schedule (20240108):

Date	Week	Topic	
1/10	1	Introduction	
1/8-1/12	1	No labs	
1/17	2	Fundamentals of molecular and cellular biology	
1/17-1/18	2	Only Wed./Thurs. labs (MLK day): Intro to pipetting, mass measurements, safety.	
1/24	3	Gene expression and protein synthesis I	
1/22-1/25	3	Mon./Tues.: Library/database resources and discussion of individual projects	
1/31	4	Gene expression and protein synthesis II	
2/7	5	Protein structure and function	
2/14	6	Nucleic acid structure and function	
2/21	7	Introduction to specific projects I	Harris
2/28	8	Introduction to specific projects II	Eddy Angerhofer
3/6	9	Introduction to specific projects III	
3/20	10	Introduction to specific projects IV	
3/27	11	Genome editing	
4/3	12	Receptor signaling	Eddy
4/10	13	mRNA Vaccines	
4/17	14	Enzyme engineering	
4/24	15	Drug discovery	
4/22-4/23	15	Mon. and Tues: Student presentations	

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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	Nicole Alejandra Colon Rosa email: ncolonrosa@chem.ufl.edu
Rudolf	U103	Tel.: 294-7221 (office, SFH302G) email: jrudolf@chem.ufl.edu O.H.: Monday 9–10 am in SFH302G	Xiuting Wei email: weixiuting@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	Mahi Athar email: athar.uzafar@ufl.edu
Eddy	U106	Tel.: 294-1048 (office, SFH302C) email: matthew.eddy@chem.ufl.edu O.H.: TBA in SFH302C	Sreyashi Das, email: sr.das@chem.ufl.edu
Harris	U107	Tel: 352-932-1742 (office, SFH302F) email: harris@chem.ufl.edu O.H.: Monday 9–10 am in SFH302F	Nidhi Kalia email: nidhi.kalia@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: 1/17 (MLK Day), 3/11 – 3/15 (Spring Break), 4/25–4/26 (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.

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

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. Students will learn aspects of DNA purification and analysis, protein expression and quantification, enzyme purification, biophsyical characterization, chromatography, electrophoresis, immunological techniques, and spectroscopic analysis. Students will design their own experimental procedures to address a research question that they will develop, continually analyze, evaluate, and report on. They 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:

Carboxylesterases (Butcher): Acyl-CoA oxidases (ACOXs). 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.

Enzymes from Marine Organisms (Bruner): This project is part of a multi-university team's efforts to discover novel anticancer natural product small molecules from underexplored marine sources: coral sponges and associated cyanobacteria. Samples have been collected and analyzed using high throughput DNA sequencing of the associated metagenomes. In this project we will employ AI-based genome mining approaches to identify protein machinery encoding for the production of prioritized small molecules. After the selection of gene products, we will combine a variety of approaches to characterize substrate scope and chemical mechanism of pathway enzymes, providing insight into function and pathway chemistries. Structural biology will be combined with mechanistic enzymology to detail new enzyme chemistry. Enzyme structure determination with X-ray crystallography is a valuable and useful approach to assign function, gain insight into the mechanism and guide inhibitor design/protein engineering. Our initial focus is on enzymes predicted to play a role in the installation of structurally unique chlorine atoms as the final step in the production of anticancer anaenamides.

Terpene synthases (Rudolf): Terpenoids and 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, enzyme products will be isolated and characterized. TSs of particular interest will be targeted for active site mutagenesis revealing the functional roles of active site amino acids.

Yeast Proteinase A Inhibitor IA_3 (Fanucci): 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 its parent organism protease. The α -helix of IA_3 resides in the N-terminal region of the protein, leaving the C-terminus structurally unresolved and disordered. To date, several IA_3 sequences from various *Saccharomyces* genera have been reported in the NCBI database, revealing that the N-terminal sequences are in general highly conserved, representing various protein-based inhibitors. Goals for this semester will be to better understand how fractional charge and charge distribution in the sequence of IA_3 impacts helical folding (via circular dichroism spectroscopy) and surface hydration (via electron paramagnetic resonance spectroscopy and Overhauser dynamic nuclear polarization spectroscopy). Cloning of the various IA_3 genes, including incorporation of site-specific cysteine substitutions for spin-labeling and hydration studies, protein overexpression, protein growth, and protein purification will be completed in this lab to then study variation in changes of hydration, dynamics and folding that are associated with altered fractional charge and charge distribution.

Oxalate Decarboxylase (Angerhofer): Oxalate Decarboxylase (OxDC) is a stress-induced enzyme in the soil bacterium *Bacillus subtilis*. It is expressed when these bacteria encounter low-pH environments. The enzyme catalyzes the redox-neutral disproportionation reaction of mono-protonated oxalate into carbon dioxide and formate. Oxalic acid, the conjugate acid of the oxalate monoanion, 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 only partially 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. Our prior work on OxDC revealed the catalytic competency of a long range electron transfer (LRET) between the N– and C–terminal Mn centers which depends on the quaternary structural organization of the enzyme. SDM will be applied to test this LRET further through modifications of the quaternary organization of the enzyme. Moreover, we will work with an isozyme of OxDC, named OxDD, which is also expressed in *Bacillus subtilis*, yet much less characterized. Students will work on expressing and purifying this protein with the goal of growing protein crystals suitable for X-ray diffraction studies in order to determine its 3-dimensional structure.

G-Protein Coupled Receptors (Eddy): (1) Biologics are proteins used as drugs to treat numerous diseases, especially cancers and inflammatory diseases. Though potent and specific, biologics are often degraded by the harsh environment inside the human body, limiting their application. One of the most promising approaches to overcoming this central challenge is through PEGylation, *i.e.*, covalently attaching a polymer such as polyethylene glycol (PEG) to the protein drug, improving its robustness. However, there exists no rational approach to select locations in the protein to PEGylate to maximize this benefit. In this project, students will prepare protein variants, PEGylate them, and compare the impacts of PEGylation on the functional and physical properties of the protein. This

information will be used to provide a rational approach to designing PEGylated proteins with predictable beneficial properties.

(2) 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 (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.

COVID NSP-15 Endonuclease (Harris): NSP-15 is an uridine specific endoribonuclease conserved across coronaviruses including SARS-CoV-2 that processes viral RNA to evade detection by host defense systems. Crystal and CryoEM structures of Nsp15 from different coronaviruses reveal a common hexameric assembly, yet how the enzyme recognizes and processes RNA remains poorly understood. We are investigating the oligomerization and substrate specificity of COVID NSP-15 using the tools of mechanistic enzymology and high throughput mutagenesis. Our goals are to understand its biological role and the defining principles for developing effective inhibitors as potential antiviral drugs. Recent results demonstrate that NSP-15 requires divalent metal ions to activate cleavage of oligonucleotide substrates, but does not employ them in catalysis. To test this hypothesis and set the stage for more detailed structural and biophysical studies of NSP-15 regulation, we are creating a series of mutant proteins with specific amino acid substitutions at positions identified as involved in substrate binding and catalysis. The information gained studying these mutant enzymes will not only reveal fundamentally important principles of protein specificity and catalytic mechanism, but provide design principles for the development of new antiviral agents that target NSP-15.

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. 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 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.