CHEM 462, Advanced Topics in Biochemistry

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Office Hours: Tuesdays and Thursdays between 1.00-3.00 PM. Or if you prefer, email me for a specific appointment time.

Classroom: Bilger 242 **Hours:** Tuesdays & Thursdays between 10.30-11.45 AM

Goals

Up until this point, you have learned many of the basic pathways and concepts that are relevant to the cellular metabolism of all species. These include the utilization of carbohydrates and fatty acids for energy, the storage of energy in carbohydrates and fatty acids, the metabolism of amino acids, the structure and role of proteins, the biosynthesis of lipids and biological membranes, and some very basic aspects of the coordination of these activities. Depending on the other courses you have taken, you may have learned some aspects of genetics and the storage of protein sequence information in the DNA sequence of every organism and the central dogma.

The first goal of this course is to cover advanced topics not covered in a traditional Biochemistry course, including:

1. DNA and RNA. The structure, chemistry, and function of nucleic acids.

2. DNA Replication and DNA Repair. Maintaining the fidelity of the DNA sequence and passing this sequence on to future generations is central to sustained life. **DNA Transcription and the many uses of RNA.** DNA sequence is converted to RNA, which codes for various proteins, but can also have many other purposes within the cell. **RNA Translation and Protein Synthesis.** Ribosomes are huge RNA-based enzymes that are capable of reading the RNA sequence and generating proteins with very specific amino acid sequences.

3. Regulation of Gene Expression. Gene expression can be regulated at many different levels and by many different methods, including direct control through protein binding to DNA or RNA, and indirect control (epigenetics) through chemical modification of nucleic acids or proteins bound to DNA. There are also examples of gene regulation at the RNA level.

4. Eukaryotic gene editing, CRISPR/Cas9. Is a technology with the ability to change an organism's DNA *in vivo*, i.e. a type of genetic engineering in which DNA is inserted, deleted, modified or replaced in the genome of living organisms.

5. The life of a protein, from synthesis to degradation. Protein activity is regulated by serval factors such as the rate of protein synthesis and proteasomal degradation, allosteric transitions, and chemical modifications called post-translational modifications.

6. Bioinformatics and how to use bioinformatics tools like BLAST, ClustalW2, and Sequence Logo Plot. Bioinformatics is an interdisciplinary filed that develops methods and software tools for understanding biological data. Common uses of bioinformatics include the identification of candidate genes and single nucleotide polymorphisms. Often, such identification is made with the aim of better understanding the genetic basis of disease, unique adaptations, desirable properties, or differences between populations.

7. Regulation of Enzyme Function. Enzyme activity can be regulated directly, either through binding of various biomolecules or proteins, or through chemical modification, especially phosphorylation of specific amino acid side-chains.

8. Hormones and Control of Mammalian Pathways. In organisms that have multiple organs and tissues, the function of these tissues must be coordinated to benefit the organism. The primary method of control is through the release, binding, and exerted action of various types of hormones and neurotransmitters.

The second goal of this course is to introduce you to how scientists advance the field of biochemistry through incremental improvements in our knowledge of very specific topics. We don't just wake up one day with a fully formed understanding of how things work. Based on prior studies and theories, we acquire knowledge and experience to formulate various hypotheses, decide which ones are worth testing, and devise various types of experiments that can hopefully unambiguously prove or disprove a hypothesis. In this class you will acquire knowledge from the lectures, the book and from research articles summarized into the final research project writing a review style manuscript.

I will treat this class as a "research group" and we will study three research topics to begin to look at how scientists:

- approach a problem
- learn an appropriate amount of background information to understand the relevant issues
- ask questions about how the system works and formulate hypotheses and aims
- design experiments with appropriate controls to test the hypotheses

In the final month of the course, you will pick an area of research you find most interesting, study the literature to arrive at some understanding of the current state of knowledge, and prepare a written research manuscript that will organize your hypotheses and previous published knowledge in the field. This will be written in the style mini-review article.

Text book: Lehninger: Principles of Biochemistry, 6th Edition
Authors: Daniel L. Nelson and Michael M. Cox, Publisher: W.H. Freeman & Co., New York, NY
We are using this textbook because it was already used for BIOL 402. Much of the information content is excellent, but the images are not particularly interesting. We will jump around and cover roughly 9 chapters in this book.

I will also occasionally be handing out papers and photocopies of pages from other textbooks and online journals. Important: Please read the reading assignments before class, I will assume that you have.

Lectures & Discussions

- ✓ The class will be divided into 5 Modules. The class will be taught by Dr. Ellinor Haglund (the main instructor) and two guest lecturers.
- ✓ Most lectures will be based on the textbook or review articles.
- ✓ Lectures will be very informal. I want this to be more like a group meeting where we come to a collective understanding of how the experimental system works.
- ✓ We will also have instructor and student-led discussions of journal articles.

Homework, Quizzes & Exams

- ✓ Reading assignments will be given prior to each lecture.
- ✓ There will be short quizzes throughout the class. Typically, the quiz will be short answer questions that will take ~15-20 min.
- ✓ There will be 3 exams throughout the semester.

Writing Assignments

- ✓ **Students must adequately complete all writing assignments to pass the course with a D grade or better. Students who do not complete all writing assignments will get a D- or an F.**

- ✓ Students will prepare a research manuscript written as a review article based upon an independent research idea. This idea can be based on journal articles or the work of a specific scientist but should be a new idea or concept that extends the current published work. The final manuscript must be at least 12 pages, including figures and references (12 pt font, 1.5 line spacing)
- ✓ Draft versions of each component of the manuscript will be turned in or uploaded to Laulima with specific due dates.
- ✓ Feedback on content and writing style will be provided:
  - Project summary (abstract) and specific aims – email feedback from the instructor
  - Intro and Background – peer review from another student
  - Summery and Future directions– in-class writing workshops studying successful publications in Nature/Science
- ✓ The final version of the manuscript will be due in class on the last day of the semester.

Grading:

- ✓ Quizzes worth 20% total.
- ✓ Two midterm exams each worth 20% of the final grade each.
- ✓ There will be an exam focusing on previous knowledge about the central dogma, i.e. from DNA-to-proteins the first week pf the semester (10% of the final grade, curved at a B average).
- ✓ Review article worth 30% of the final grade.
  - Hypothesis & Abstract (draft), 5%
  - Specific Aims (draft), 5%
  - Intro and background (draft) for peer review (at least 5 pages), 5%
  - Final version, 15%
- ✓ The finished version of the manuscript will serve as your final project and will be due on the last day of class. There will be NO final exam.

Student Learning Outcomes:

- ✓ Students will understand the chemical principles that underlie DNA replication and transcription, and RNA translation and protein synthesis.
- ✓ Students will understand the interplay between methods for regulating networks of biochemical reactions, including genetic regulation, hormones and signal transduction, and protein activation and inhibition.
- ✓ Students will understand how biochemical reactions can be described at an atomic level, including how enzyme catalysts and cofactors can accelerate difficult reactions.
- ✓ Students will use literature search processes to gain knowledge of recent advances in biochemistry, develop a hypothesis-driven research proposal, summarized as a review article.

Attendance and Participation:

- ✓ Attendance at class sessions is expected

Academic Misconduct:

- ✓ UH Manoa Student Conduct code Executive Policy 7.208. IV.B.1a "Acts of dishonesty, including but not limited to the following: Cheating, plagiarism, or other forms of academic dishonesty. Cheating is an act of academic dishonesty and includes, but is not limited to: (1) use of any unauthorized assistance in taking quizzes, tests, or examinations; (2) use of sources beyond those authorized by the instructor in writing papers, preparing reports, solving problems, or carrying out other assignments; (3) the acquisition, without permission, of tests or other academic material belonging to a member of the UH faculty, staff or student body; and (4) engaging in any behavior specifically prohibited by a faculty member in the course syllabus or class discussion.

- ✓ Plagiarism is also an act of academic dishonesty and includes, but is not limited to, the use, by paraphrase or direct quotation, of the published or unpublished work of another person without full and clear acknowledgement. It also includes the unacknowledged use of materials prepared by another person or agency engaged in the selling of term papers or other academic materials."
- ✓ Plagiarism penalty: First offense, students will be asked to re-write the report without the offending material and will be given lower credit(s) if they do so. Subsequent offense: student will receive zero credit and the matter will be referred to the Department Chair and Dean for further action.
- ✓ Likewise, cheating on an exam will be dealt with severely. A student will receive no credit for the entire exam, and the offense will be reported to the Department Chair and Dean for further action.

Exp#		Topic
	1/17	Introduction to LDH experiment; General lab safety; biological safety Buffer preparation, method development for LDH purification
1-1	1/24	Purification of LDH from chicken breast tissue; ammonium sulfate precipitation
1-2	1/31	Purification of LDH; affinity chromatography
1-3	2/7	Bradford's Assay for determining protein concentration and SDS PAGE analysis of LDH First writing workshop and data discussion
1-4	2/14	9/17 FIRST DRAFT DUE for LDH report (introduction and experimental sections)` Direct spectrophotometric assay to determine K_M (NAD^+) and k_{cat}
1-5	2/21	9/24 RETURN First Draft of LDH Indirect coupled assay to determine K_M (lactate)
1-6	2/28	Determination of K_i for oxamate and oxalate 10/1 Second writing workshop and data discussion
2-1	3/6	10/3 SECOND DRAFT DUE for LDH report (All sections) Transform BL21(DE3) cells with S6 DNA Bioinformatics on DNA sequence
	3/13	10/10 Return Second Draft of LDH Lecture on protein stability using CD and fluorescence
	3/20	NO CLASS-SPRING BREAK

	3/27	Mixing GdmCl and Eq titration
2-3	4/3	10/22 FIRST DRAFT of S6 (introduction and experimental sections) 10/29 FINAL DRAFT DUE for LDH report 10/29 Return First Draft of S6 CD/Fluorescence Thermal melt Writing workshop and data discussion
	4/10	NO CLASS-GOOD FRIDAY
	4/17	SECOND DRAFT of S6 report due (all sections) Final exam review Writing workshop and data discussion Return Final Draft of LDH
	4/24	Return Second Draft Final Exam Writing workshop and data discussion
	5/1	FINAL DRAFT of S6 report due

*Experiment
Lecture
Due Date

Category	Excellent	Average	Poor	Score
Abstract	10-9 ● Abstract Explains the importance of the experiments.. ● Makes the reader want to read the paper. ● Summarizes the data found.	8-7 ● Abstract does not grab the attention of the reader. ● Has some information on the purpose of the experiments. ● Summarizes some of the data found	6-0 ● Abstract does not grab the attention of the reader. ● Does not have the purpose of experiments. ● Does not have data.	___/10
Introduction	25-23 ● Gives relevant background on the protein of interest. ● Explains the broader impact this research gives. ● Explains what experiments were conducted.	22-20 ● Has some background on the protein of interest. ● Describes little of the broader impacts ● Has some information on the experiments conducted.	19-0 ● Gives no background on the protein of interest. ● Introduces little about experiments conducted. ● Has no broader impacts.	___/25
Methods	40-36 ● Speaks in third person past tense consistently. ● Uses scientific wording to convey how experiments were conducted including reagent concentrations.	35-32 ● Speaks in third person past tense some of the time. ● Gives some of the important steps in series of experiment. Includes some of the concentrations used.	31-0 ● Does not speak in third person past tense. ● Gives little methods used in the series of experiment. Does not give any concentrations of reagents used.	___/40
Results	20-18 ● Clearly states the data in an organized way. ● Has relevant graphs to represent the data with clear legends.	17-16 ● Includes some of the data obtained. ● Has graphs to represent data.	15-0 ● Does not include all of the data obtained. ● Has graphs that do not clearly represent the data. Or skews the data.	___/20
Discussion	40-36 ● Discusses all of the relevant data that was mentioned in the Results section. ● Clearly explains what the data means and what implications it may have.	35-28 ● Discusses some of the data that was mentioned in the Results section. ● Explains what the data means and what implications it may have.	27-0 ● Does not discuss all of the relevant data that was mentioned in the Results section. ● Does not explain what the data means and what implications it may have.	___/40
Revisions	20-18 ● Critiques have been used in making changes.	16-15 ● Some critiques have been used in making changes.	14-10 ● Little to no critiques were used in editing this paper.	___/20
Reference	5-4 ● Sources were relevant and used well to support the argument of the paper.	3-2 ● Sources were used for the argument of the paper.	1-0 ● Sources were not relevant and/or does not support the argument of the paper.	___/5
Overall	10-9 ● The paper was excellent and could be used as an example. ● Spelling and grammar were used well. Captured the audience's attention and kept it. ● Formatting is clean and easy to follow.	8-7 ● The paper was okay, but needs a little work. ● Okay spelling and grammar. ● Formatting is okay.	6-3 ● The paper needs a lot of work. ● Needs help with spelling and grammar. ● Formatting has no system.	___/10