BIOCHEMISTRY 551: BIOCHEMICAL METHODS SYLLABUS

Course Description: Biochemistry 551 is an integrated lecture, lab and seminar course that covers biochemistry-centered theory and techniques. The course is designed for upper-level undergraduate students majoring in Biochemistry. Students learn how to apply a broad range of biochemical, genetic, and physical techniques to modern biochemical research. Students also learn how to analyze and interpret the primary scientific literature, develop an understanding of the communication of data, and extrapolate biochemical techniques to basic research.

Lectures introduce concepts and theory that are subsequently explored in detail in experiments. The experiments are designed to provide hands-on experience with instruments and techniques that are used in modern biochemical research. The curriculum incorporates a small research project beginning with the PCR amplification and cloning of the *HCAII* gene, which codes for human carbonic anhydrase II (HCAII). As the semester progresses, students overexpress, purify and assay wild type and mutant HCAII protein. Experiments focus on instrumental techniques including PCR, spectrophotometry, gel electrophoresis, protein overexpression and purification, enzyme assays and fluorescence spectroscopy.

Learning Objectives: By the end of Biochemistry 551, students should be able to:
1. Apply the fundamental concepts of experimental design to answer a scientific question
2. Explain the theory of several fundamental biochemical techniques
3. Perform experiments and collect and analyze the data
4. Identify potential problems that arise during experiments and develop solutions
5. Interpret the analyzed data to determine the results of the experiment
6. Communicate experimental results in both oral and written reports
7. Critically analyze data in peer-reviewed scientific publications

Readings:
There is no textbook for this course; however, you must purchase a 551 Lab Manual from the DoIT Tech Store.

Additionally, as part of the seminar component of the course you will be required to read one paper from the primary literature each week. During the first week of class you will be assigned a seminar section and topic. You will then be provided a list of the papers you will be reading over the semester.

Weekly Course Meeting Times:
Mon 11-11:50: Seminar in rooms TBA
Wed 11-11:50: Lecture in Biochem 1120
Fri 11-11:50: Pre-Lab Lecture in Biochem 1120
Lab sections:
301: Mon 12:20-4:00
302: Tues 12:20-4:00
303: Wed 12:20-4:00
304: Thurs 9:00-12:20

Assignments

Assignment	Points per assignment	Total Points
Exams (2)	150	300
Lab performance		100
Pre-lab quizzes (10)	5	50
Mini lab reports (10)	10	100
Final lab report		150
Oral lab report		100
Literature seminar		150
Literature seminar participation		50
Total		1000

Grading Scale

Points	Grade
> 900	A
850–900	AB
800–849	B
750–799	BC
700–749	C
600–699	D
< 599	F

NOTE: Students with disabilities who need accommodation are encouraged to meet with the instructors as soon as possible.

Course Schedule:

Wed. Jan 21	Lecture 1: Class overview and description of HCAII (Dr. Lynne Prost)
Fri. Jan 23	Lecture 2: Protein structure and computational analysis (Dr. Prost)
Mon. Jan 26	*Seminar: How to give a scientific presentation (Dr. Prost)*
Jan 26 - 29	Lab 1: Computational analysis of HCAII - Analyze protein structure - Each section selects 2 potential mutations
Wed. Jan 28	Lecture 3: PCR (Dr. Prost)
Fri. Jan 30	Pre-lab 2: PCR (Karl Wetterhorn)
Mon. Feb 2	*Seminar: Instructor Seminar*
Feb 2 - 5	Lab 2: PCR amplification of the *HCAII* gene - Check-in and pipette workshop - PCR amplification of *HCAII*
Wed. Feb 4	Lecture 4: Cloning and mutagenesis (Dr. Prost)
Fri. Feb 6	Pre-lab 3/4: Analysis of *HCAII* PCR product AND Ligation and transformation (Ti-Yu Lin)
Mon. Feb 9	*Student seminar 1*
Feb 9 - 12	Lab 3: Analysis of the *HCAII* PCR product - Analysis and purification of PCR product - Restriction digest of PCR product and vector
Wed. Feb 11	Lecture 5: Control of recombinant overexpression of proteins (Dr. Prost)

Fri. Feb 13	Scientific writing (Dr. Prost)
Mon. Feb 16	*Student seminar 2*
Feb 16 - 19	Lab 4: Ligation of *HCAII* into pET28b vector - Purify restriction digests - Ligate *HCAII* insert into vector - Transform the ligations into *E. coli* DH5α
Wed. Feb 18	Lecture 6: Protein expression systems (Dr. Prost)
Fri. Feb 20	Pre-lab 5/6: Screening pET28b-*HCAII* clones AND Induction of HCAII expression (Clay Williams)
Mon. Feb 23	*Student seminar 3*
Feb 23 - 26	Lab 5: Screening for pET28b-*HCAII* clones - Students inoculate bacterial cultures prior to lab period - Miniprep DNA - Screen by restriction digest - Transform clones into *E. coli* RB DE3
Wed. Feb 25	Lecture 7: Gel electrophoresis of biomolecules (Dr. Prost)
Fri. Feb 27	Exam review
Mon. Mar 2	*Student seminar 4*
Mar 2 - 5	Lab 6: Induction of His-tagged HCAII expression - Induce cultures with IPTG - Collect samples for analysis - Measure cell growth - Pellet culture for purification
Wed. Mar 4	**EXAM 1 on lectures 1 – 7**
Fri. Mar 6	Pre-lab 7: Purification of HCAII (Kim Haupt)

Mon. Mar 9	*Student seminar 5*
Mar 9 - 12	Lab 7: Purification of wild type and mutant HCAII - Cell lysis - Ni column
Wed. Mar 11	Lecture 8: Protein purification (Dr. Prost)
Fri. Mar 13	Pre-lab 8: Analysis of His-tagged HCAII purification (Shane Bernard)
Mon. Mar 16	*Student seminar 6*
Mar 16 - 20	Lab 8: Analysis of His-tagged HCAII expression and purification - Pour SDS-PAGE gels - Analysis of HCAII expression by electrophoresis - Spectroscopic determination of concentration
Wed. Mar 18	Lecture 9: UV/Vis and Fluorescence spectroscopy (Dr. Prost)
Fri. Mar 20	Pre-lab 9 session: Intrinsic tryptophan fluorescence (Danielle Hamm)
Mon. Mar 23	*Student seminar 7*
Mar 23 - 27	Lab 9: Intrinsic tryptophan fluorescence - Measure stability of wt and mutant protein
Wed. Mar 25	Lecture 10: Protein folding (Dr. Prost)
Fri Mar 27	Pre-lab 10/11 session: Enzymatic activity of HCAII (Katharine Schulz)
Mar 30 – Apr 3	SPRING BREAK
Mon. Apr 6	*Student seminar 8*

Apr 6 - 9	Lab 10: HCAII enzyme activity - Measure enzymatic activity of wild type and mutant HCAII
Wed. Apr 8	Lecture 11: Enzyme kinetics (Dr. Prost)
Fri. Apr 10	Pre-lab 11 session: FRET to detect ligand binding (Emily Garnett)
Mon. Apr 13	*Student seminar 9*
Apr 13 - 16	Labs 11/12: HCAII enzyme and inhibitor assays and FRET - Half the class will measure the ability of a small molecule to inhibit HCAII enzymatic activity - Half the class will use FRET to detect ligand binding to wild type and mutant HCAII
Wed. Apr 15	Lecture 12: Ligand binding (Dr. Prost)
Fri. Apr 17	Guidelines for Oral Report, Final Report, and Troubleshooting Lab (Dr. Prost)
Mon. Apr 20	*Student seminar 10*
Apr 20 - 23	Labs 11/12: HCAII enzyme and inhibitor assays and FRET - Half the class will measure the ability of a small molecule to inhibit HCAII enzymatic activity - Half the class will use FRET to detect ligand binding to wild type and mutant HCAII
Wed. Apr 22	Lecture 13: Emerging biochemical techniques (Dr. Prost)
Fri. Apr 24	Lecture overflow
Mon. Apr 27	*Student Seminar 11 (if necessary)*
Apr 27 - 30	Troubleshooting Lab: Students have the opportunity to repeat one lab of their choice.
Wed. Apr 29	Exam review

Fri. May 1	**EXAM 2 on lectures 8 - 13**
Mon. May 4	*Student Seminar 12 (if necessary)*
May 4 - 7	**Group Oral Reports** (Rooms TBD)
Wed. May 6	NO CLASS
Fri. May 8	NO CLASS
Mon. May 11	**LAB REPORT DUE**