

Biochemistry 651 Syllabus: Fall 2014

Course Description: Biochemistry 651 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 *hcall* gene, which codes for human carbonic anhydrase II (HCAII). As the semester progresses, students overexpress, purify and assay 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 651, 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

Lab Sections:

304 - Monday: 1:20 – 5:00 pm

301 - Tuesday: 1:20 – 5:00 pm

302 - Wednesday: 1:20 – 5:00 pm

303 - Thursday: 9:00 – 12:40 pm

Room Locations:

Lectures will take place in room 1120 Biochemistry (the building at 420 Henry Mall).

Labs will take place in room 2118 Biochemistry, with the exception of lab 1 which will take place in room 2530 Microbial Science Building.

During the first week of class, you will be assigned a seminar and a seminar room.

Course Schedule:

Wed. Sept 3	Lecture 1: Class overview and description of HCAII (Prof. Alessandro Senes, Dr. Lynne Prost)
Fri. Sept 5	Lecture 2: Protein structure and computational analysis (Prof. Senes)
Mon. Sept 8	<i>Seminar: How to give a scientific presentation (Dr. Prost)</i>
Sept 8 - 11	Lab 1: Computational analysis of HCAII <ul style="list-style-type: none">- Analyze protein structure- Each section selects 2 potential mutations
Wed. Sept 10	Lecture 3: PCR (Dr. Prost)
Fri. Sept 12	Pre-lab 2 lecture: PCR (TA)
Mon. Sept 15	<i>Seminar: Instructor Seminar</i>
Sept 15 - 18	Lab 2: PCR amplification of the <i>hcall</i> gene <ul style="list-style-type: none">- Check-in and pipet workshop- PCR amplification of <i>hcall</i>
Wed. Sept 17	Lecture 4: Cloning and mutagenesis (Dr. Prost)
Fri. Sept 19	Pre-lab 3/4 lecture: Analysis of <i>hcall</i> PCR product AND Ligation and transformation (TA)
Mon. Sept 22	<i>Student seminar 1</i>
Sept 22 - 25	Lab 3: Analysis of the <i>hcall</i> PCR product <ul style="list-style-type: none">- Analysis and purification of PCR product- Restriction digest of PCR product and vector
Wed. Sept 24	Lecture 5: Protein expression systems (Dr. Prost)

Fri. Sept 26	<p>Scientific writing (Dr. Prost)</p> <ul style="list-style-type: none"> - Read the sample lab reports and come to class prepared to discuss
Mon. Sept 29	<i>Student seminar 2</i>
Sept 29 – Oct 2	<p>Lab 4: Ligation of <i>hcaII</i> into pET28b vector</p> <ul style="list-style-type: none"> - Purify restriction digests - Ligate <i>hcaII</i> insert into vector - Transform the ligations into <i>E. coli</i> DH5α
Wed. Oct 1	Lecture 6: Control of recombinant overexpression of proteins (Prof. Senes)
Fri. Oct 3	Pre-lab 5/6 lecture: Screening pET28b- <i>hcaII</i> clones AND Induction of HCAII expression (TA)
Mon. Oct 6	<i>Student seminar 3</i>
Oct 6 - 9	<p>Lab 5: Screening for pET28b-<i>hcaII</i> clones</p> <ul style="list-style-type: none"> - Students inoculate bacterial cultures from best plate prior to lab period - Miniprep DNA - Screen by restriction digest - Transform clones into <i>E. coli</i> RB DE3
Wed. Oct 8	Lecture 7: Gel electrophoresis of biomolecules (Prof. Senes)
Fri. Oct 10	Exam review
Mon. Oct 13	<i>Student seminar 4</i>
Oct 13 - 16	<p>Lab 6: Induction of His-tagged HCAII expression</p> <ul style="list-style-type: none"> - Induce cultures with IPTG - Collect samples for analysis - Measure cell growth - Pellet culture for purification
Wed. Oct 15	EXAM 1 on lectures 1 – 7

Fri. Oct 17	Pre-lab 7 lecture: Purification of HCAII (TA)
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Mon. Oct 20	<i>Student seminar 5</i>
Oct 20 - 23	Lab 7: Purification of wild type and mutant HCAII - Cell lysis - Ni column
Wed. Oct 22	Lecture 8: Protein purification: part 1 (Prof. Senes)
Fri. Oct 24	Pre-lab 8 lecture: Analysis of His-tagged HCAII purification (TA)
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Mon. Oct 27	<i>Student seminar 6</i>
Oct 27 - 30	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. Oct 29	Lecture 9: Protein purification: part 2 (Prof. Senes)
Fri. Oct 31	Pre-lab 9 lecture: Intrinsic tryptophan fluorescence (TA)
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Mon. Nov 3	<i>Student seminar 7</i>
Nov 3 - 6	Lab 9: Intrinsic tryptophan fluorescence - Measure stability of wt and mutant protein
Wed. Nov 5	Lecture 10: UV/Vis spectroscopy (Prof. Senes)
Fri Nov 7	Pre-lab 10 lecture: Enzymatic activity of HCAII (TA)
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Mon. Nov 10	<i>Student seminar 8</i>
Nov 10 - 13	Lab 10: HCAII enzyme activity - Measure enzymatic activity of wild type and mutant HCAII
Wed. Nov 12	Lecture 11: Fluorescence (Prof. Senes)

Fri. Nov 14	Pre-lab 11 lecture: FRET to detect ligand binding (TA)
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Mon. Nov 17	<i>Student seminar 9</i>
Nov 17 - 20	Lab 11: FRET - Use FRET to detect ligand binding to wild type and mutant HCAII
Wed. Nov 19	Lecture 12: Enzyme assays and protein folding (Prof. Senes)
Fri. Nov 21	Guidelines for Oral Report, Final Report, and Troubleshooting Lab (Dr. Prost)
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Mon. Nov 24	<i>Student seminar 10</i>
Nov 24 - 27	NO LAB THIS WEEK
Wed. Nov 26	NO CLASS
Fri. Nov 28	NO CLASS
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Mon. Dec 1	<i>Student Seminar 11 (if necessary)</i>
Dec 1 - 4	Troubleshooting Lab: Students have the opportunity to repeat one lab of their choice.
Wed. Dec 3	Lecture 13: Emerging Biochemical Technologies AND Exam Review
Fri. Fri Dec 5	EXAM 2 on lectures 8 - 13
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Mon. Dec 8	<i>Student Seminar 12 (if necessary)</i>
Dec 8 - 11	Group Oral Reports (Rooms TBD)
Wed. Dec 10	NO CLASS

Fri. Dec 12

NO CLASS

Mon. Dec 15

LAB REPORT DUE