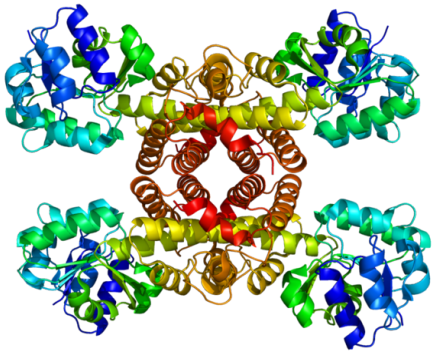
Chemistry 371: Advanced Biochemistry Laboratory

Course description

During the past century scientists have developed the technology to study biology with increasingly fine resolution. Biochemistry is the study of biology at molecular resolution. In this advanced laboratory course, students will learn to use modern biochemical techniques to isolate, purify, and characterize the enzyme 3-hydroxyisobutyrate dehydrogenase from Arabidopsis thaliana (AtHDH1). Additionally, students will generate mutations within and near the active site of the enzyme and measure changes in catalytic function. Your ultimate goal will be to determine which amino acid, if any, are important for enzyme function.



3-hydroxyisobutyrate-dehydrogenase
(H. sapiens)

Instructors and Course Resources

Instructor for weeks 1-7: Prof. Matthew Rouhier
Email: rouhierm@kenyon.edu
Office: 208 Tomsich Hall
Office Hours: Monday 1-2pm, Wednesday 4-5pm, Thursday 10-11am and by appt.

Instructor for weeks 8-14: Prof. Kerry Rouhier
Email: rouhierk@kenyon.edu
Office: 212 Tomsich Hall
Office Hours: Monday 1-3pm, Tuesday 4-5pm, Friday 2-3pm and by appt.

Class location:	Tomsich 203 (with pre-lab discussion in Tomsich 206)
Class time:	1:10-4:00 pm Tuesday (section .01) and Wednesday (section .02)
Materials:	Computer, calculator, and associated literature posted to Moodle
Websites:	*moodle.kenyon.edu* and *benchling.com*

What to expect

From the course – to be challenged in ways that require you to think creatively and analytically. Each experiment builds upon previous results and supports the work completed in subsequent weeks, much the way research is typically performed. You will read primary literature and carry out a bioinformatics analysis to help you form hypotheses and validate your results along the way. Much of the data collected this semester will be novel and could lead to a future publication in a peer-reviewed journal. You can expect to learn many common biochemical techniques, such as how to purify protein and characterize enzymes. Your results will be summarized in a manuscript-style report that could be used in future publications. This exercise will help you fine tune your scientific writing skills.

From the instructor – to treat each person with respect, arrive to class on time and prepared, return graded and assessed items in a timely manner, and be available outside of class for questions or further discussion during office hours or other scheduled meetings.

Of the students – to respect others, be on time (when arriving to class, turning in assignments, etc.), and be prepared for class (read the background material, notebook up-to-date, and ready to perform the experiment).

Course Goal and Objectives

Kenyon College has a list of goals and objectives for every graduating student that reflects the College's commitment to a liberal arts education. Additionally, the Chemistry Department and the American Society for Biochemistry and Molecular Biology have learning goals specifically for graduating majors and biochemistry and molecular biology programs, respectively. Based on their objectives, this course will broadly address: (1) the process of science, (2) the accessing, comprehending, and communication of science, and (3) the community of science. The complete list of learning goals (College, Department, Society) can be found at the end of this syllabus.

Course Policies

Academic Honesty: Kenyon College is, at the core, an intellectual community of scholars – students and faculty – engaged in the free and open exchange of ideas. Critical to this lively exchange and deep engagement with ideas is the academic integrity of our work, both inside and outside the classroom.

At Kenyon we expect all students, at all times, to submit work that represents these standards of academic integrity. It is the responsibility of each student to learn and practice the proper ways of documenting and acknowledging those ideas and words you have drawn upon (*see Academic Honesty and Questions of Plagiarism in the Course Catalog*). Ignorance and carelessness are not excuses for academic dishonesty. Students will be working in teams to meet both laboratory and writing goals for this class. In all cases, your work must be your own. If you are uncertain about the expectations for academic honesty in this class, please ask for clarification.

Accommodations – Students who anticipate a need for accommodations in this course because of the impact of a learning, physical, or psychological disability are encouraged to meet with me privately early in the semester to discuss their concerns. In addition, students must contact Erin Salva, Director of Student Accessibility and Support Services (740-427-5453 or salvae@kenyon.edu), as soon as possible, to verify their eligibility for reasonable academic accommodations. Early contact will help to avoid unnecessary inconvenience and delays. No accommodations of any kind will be given in this course without notification from the Coordinator of Disability Service at least one week prior to the special accommodations.

Attendance – *Completion of all laboratory sessions is mandatory.* If you know that you will miss a laboratory session for a scheduled event please contact the instructor immediately. We reserve the right to expel students from this course for excessive unexcused absences. A number of the experiments may require lab work outside of normal class hours.

Late work – There are a lot of due dates for a variety of assignments in this class. This is so that you are able to make consistent progress throughout the semester. Occasional late submissions will be accepted without penalty; however habitual offenders will start receiving a penalty of 15% per 24 hours late (including weekends). Students with frequently late submissions will be notified of the enforcement of the penalty after 3 late submissions.

Responsibility – As members of the Kenyon College Faculty, we are concerned about the well-being and development of our students and are available to discuss any concerns. However, we want you to know that faculty members are legally obligated to share certain information with the College's Title IX coordinator. This is to ensure the student's safety and welfare is being addressed, consistent with the requirements of the law. These disclosures include, but are not limited to, reports of sexual assault, relational/domestic violence, and stalking.

Technology use – Students will need to bring a laptop to each class meeting to access the electronic notebook and the course Moodle page. You may use your own or laptops can be provided with advanced notice. Please refrain from using laptops to email, play games, or any other activity unrelated to class. For your safety, cell phones are only to be used in case of an emergency. Please have them turned off or set to silent during class. Please bring a calculator to each class.

Course Details

Experimental information – Similar to research, this course does not have a traditional lab manual. We will be using related research articles and associated documents (e.g. manufacturer's instructions) to guide our experimental steps. All pertinent information will be made available through the class Moodle site and/or in class. You must be flexible in lab to accommodate errors, unexpected delays, and adjustments to protocols. In some cases you may need to repeat a procedure to obtain acceptable results. We will adjust the schedule if needed. If you plan ahead each week for your lab work, you will be able to complete all the experiments in the time provided.

Grade – grades will be determined using the following absolute scale (A/A-): 90-100%; B(+/-): 80-89%; C(+/-): 70-79%; D(+/-): 60-69%; F: <60%). The instructors reserve the right to alter this grading scale.

Laboratory notebooks	10%	Grant proposal: draft	5%
Lab work and Pre-lab presentations	20%	Grant proposal: peer review	10%
Sequencing assignment	10%	Grant proposal: final	15%
Grant proposal: pre-proposal	15%	Enz. Kinetics Presentation	15%

Laboratory notebooks – Students will maintain a research-style electronic laboratory notebook on benchling.com. Defining characteristics of a good laboratory notebook is that someone trained in the field could repeat the experimental work using only the notebook and that you can easily write about your work months later. *Rigorous documentation of activities in the laboratory is an essential component of experimental science*. Templates have been provided for you to help guide your entries in Benchling. You will be working with a partner for the experimental work in this course and thus sharing a notebook, so it is expected that you will divide responsibilities evenly. Notebook entries will be checked twice each week: once for preparation and a second time for completion. The Purpose, Materials, and Procedure sections must be completed by 12 pm on the day of lab. The Data, Results, Figures, and Conclusion sections must be completed by the following Thursday at 5 pm unless noted otherwise.

Pre-lab presentations – Each week, 2-3 students will be randomly selected to summarize the experimental goal and procedure for the day. This summary will catalyze a brief discussion regarding the experimental details with the entire class. The presenting students should be able to answer these questions: (1) What is today's experimental goal? (2) How will you accomplish that goal? and (3) How will you know you were successful? (if applicable). Responses will be scored as satisfactory or unsatisfactory; unsatisfactory responses will be discussed with the student during the subsequent lab session.

Grant proposal – One of the goals of this course is to help you enhance and fine tune your scientific writing skills by assembling and writing a NSF-style grant proposal based on the proposed mutagenesis work completed in the laboratory. The proposal will be prepared in three stages: pre-proposal, full proposal draft with a peer-review, and the final full proposal. Each document will be due by Friday, 5 pm on the dates listed below.

Sequence work – It is important to have a thorough understanding of HDH1 (sequence, structure, function, etc.) in order to carry out the experiments outlined in this course and write a strong grant proposal. Therefore, students will investigate various aspects of HDH1 by reading primary literature and using various bioinformatics tools. The "sequence work" will be completed over the course of three weeks at the beginning of the semester (see due dates listed below in "Schedule"). Instructions for each assignment are described on the course Moodle page. Information gathered through these exercises will be recorded in your Benchling notebook.

Pre-proposal – As is the case with many granting programs, researchers are asked to submit a pre-proposal before being invited to submit a full research proposal. This pre-proposal summarizes the significance of the work while also providing a summary of the proposed work. To prepare for writing the pre-proposal, students will complete a series of exercises related to sequence and structure similarities. This one-page document is due early in the semester to "catalyze" the acquisition of information about HDH1. The format of the pre-proposal must follow these guidelines: 11 pt, Times New Roman font with 1 inch margins on all sides (approximately 500 words). A template is provided on the course Moodle page. The instructor will provide detailed feedback on the pre-proposal. Students should take all feedback into consideration when preparing the full proposal. The pre-proposal is due by 5pm on February 14th.

Full proposal (draft) – It is highly recommended to have major, formal documents reviewed prior to submitting for evaluation. Feedback from other experts in the field enhances the quality of the writing and increases the chances of getting funded. A draft of your full proposal will be reviewed by one or two peers prior to the final submission. The draft is due by 5pm on April 3th.

Full proposal (peer-review) – Your proposal will be reviewed by one or two peer-reviewers prior to the final submission. Each peer reviewer will be provided with a series of guided questions to help give constructive feedback. The review process will be conducted through the course Moodle site and reviewers will have one week to complete all reviews. Reviewer's comments are due by 5pm on April 10th.

Full proposal (final submission) – The final, polished proposal is due by 5pm on April 24th.

Enzyme kinetics presentation – During the final three weeks of the semester, students will analyze the function of the native and mutant enzymes via kinetic assays. Teams will prepare a presentation that provides sufficient background to understand the context for the generation of the mutant enzyme and a full analysis of the kinetic data. The presentations will take place on Friday (May 8th) of finals week.

Schedule

Class	Team	Week: Experiment	Notebook[1]	Writing assignments (due 5pm Friday of lab week)
Jan 14/15	1	1: Intro, safety, check-in, make solutions		
Jan 21/22	1	2: Native HIBDH purification (day 1)	✓	Sequence work: basic information
Jan 28/29	1	3: Native HIBDH purification (day 2)	✓	Sequence work: sequence similarity
Feb 4/5	1	4: Native HIBDH purification (day 3)	✓	Sequence work: structural similarity
Feb 11/12	1	5: Determine [protein]	✓	Grant: pre-proposal
Feb 18/19	1	6: Determine protein purity	✓	
Feb 25/26	2	7: Mutant HIBDH (site-directed mutagenesis)	✓	
SPRING BREAK!!				
Mar 24/25	2	9: Mutant HIBDH purification (day 2)	✓	
Mar 30/1	2	10: Mutant HIBDH purification (day 3)	✓	Grant: full proposal draft
Apr 7/8	2	11: Determine mutant [protein] and purity	✓	Grant: peer review
Apr 14/15	3	12: Enzyme kinetics (day 1)	✓	
Apr 21/22	3	13: Enzyme kinetics (day 2)		Grant: full proposal
Apr 28/29	3	14: Enzyme kinetics (day 3)		
Final exam week		Friday, May 8th at 8:30 am (.02) Friday, May 8th at 1:30 pm (.01)	Enzyme kinetics presentations	

[1] The Purpose, Materials, and Procedure sections must be completed by **12pm day of lab**. The Data, Results, Figures, and Conclusion sections must be completed by Thursday **at 5pm**.

* Note: this schedule may need to be adjusted time-to-time based on progress of the class. The instructor will notify the class of any changes to the schedule via email, course website, or announcements in class.

College and Departmental Learning Goals

Kenyon is institutionally committed to promoting a liberal arts education and as such has outlined the learning goals for your college-wide education that promoted and developed skills that are useful to any career but also essential for a fulfilling and valuable life. In addition, the community of students and faculty in the Chemistry Department are dedicated to achieving skills interlaced with the chemical world. The learning goals of this course are grounded on those suggested by both the college and the department. If you would like to learn more about Kenyon's learning objectives or the Chemistry Department's learning objectives, visit: https://www.kenyon.edu/directories/offices-services/registrar/course-catalog-2/administrative-matters/kenyon-college-its-mission-and-goals/ or https://www.kenyon.edu/directories/offices-services/office-of-the-provost/faculty-resources-information/department-mission-statements-and-assessment-plans/chemistry/

Chemistry Departmental Goals:

Learning Goal/Objective	Learning Assessment	Details
1. Each student should learn sufficient chemistry to serve them well in life after Kenyon.	Grant Proposal and Enzyme kinetics presentation	These two assessments serve as the culmination of the knowledge acquired and therefore serve as benchmarks for achieving sufficient chemical knowledge.
2. Each student should learn to write well by being required to answer essay exam questions, write term papers, problem set answer sheets, laboratory and research reports, all critically evaluated by faculty.	Laboratory notebook and Grant Proposal	The lab notebook is the record of your work and the map for others to build upon your work, therefore the notebook will be evaluated for completeness and clarity before and after lab work. The grant proposal serves as a means to communicate your ideas and findings to others outside your immediate field of study, therefore it will be evaluated for sound scientific understanding and clarity of thought.
3. Each student should learn effective oral communication skills by being encouraged to ask questions in all classes and converse frequently with faculty, and required to make extended oral presentations in more advanced classes as well as in the departmental Senior Capstone.	Pre-lab presentations and Enzyme kinetics presentation	Students will be prepared to answer these three questions at the start of each class: (1) What is the goal of today experiment? (2) How will you accomplish that goal? (3) How will you know you were successful? Teams will prepare a presentation that provides sufficient background to understand the context for the generation of the mutant enzyme and a full analysis of the kinetic data.
4. Each student become skilled at formulating and solving problems, both qualitative and quantitative, through the working of problem sets and exam questions and by engagement with laboratory and research projects.	Laboratory notebook and Grant Pre-proposal	Student calculate in advance the methods for preparing reagents and then will undertake those procedures during the laboratory period. The grant pre-proposal will rationalize the mutagenesis of a known protein to engineer the function kinetic properties.
5. Each student should learn to access, evaluate and use information from computerized information sources.	Sequence work and Grant proposal	The mutagenesis of HDH1 will be rationalized through comparative proteomics. Sequences for HDH1 enzymes will be acquired and analyzed through the use of publicly available databases and proteomic tools.
6. Each student should be encouraged to relate chemistry to other areas of inquiry and knowledge by enrolling in courses in other sciences, the fine arts, social sciences and humanities.	Grant proposal (pre-proposal)	The grant pre-proposal and proposal are written in the spirit of an NSF proposal. It is important that the language and explanations are broadly understood as scientists outside the proposer area of expertise may be reviewing the work.

College Goals:

Learning Goal/Objective	Learning Assessment	Details
a) Students acquire knowledge and understanding of fine arts, humanities, natural sciences, and social sciences.		
b) Students learn gather information from a variety of sources and evaluate its quality.	Sequence work and Grant proposal	The mutagenesis of HDH1 will be rationalized through comparative proteomics. Sequences for HDH1 enzymes will be acquired and analyzed through the use of publicly available databases and proteomic tools.
c) Students learn to formulate ideas rigorously and communicate them effectively, in speaking and in writing.	Grant proposal and Enzyme kinetics presentation	The grant pre-proposal and proposal are written in the spirit of an NSF proposal. It is important that the language and explanations are broadly understood as scientists outside the proposer area of expertise may be reviewing the work. Teams will prepare a presentation that provides sufficient background to understand the context for the generation of the mutant enzyme and a full analysis of the kinetic data.
d) Students learn languages and engage with diverse cultures.		
e) Students address ethical questions and make informed qualitative judgments.		
f) Students acquire quantitative skills and analyze data.	Laboratory notebook and Enzyme kinetics presentation	The lab notebook is the record of your work and the map for others to build upon your work, therefore the notebook will be evaluated for completeness and clarity before and after lab work. Teams will prepare a presentation that provides sufficient background to understand the context for the generation of the mutant enzyme and a full analysis of the kinetic data.
g) Students develop an aesthetic sensibility through practice and critical examination of the fine, performing, and literary arts.		
h) Students learn to work creatively.	Grant proposal	The mutagenesis of HDH1 is undertaken with the goal of creating a new enzyme with new activity.
i) Students learn to work collaboratively and across disciplines.	Enzyme kinetics presentation	Teams will prepare a presentation that provides sufficient background to understand the context for the generation of the mutant enzyme and a full analysis of the kinetic data.
j) Prepare for leadership and for civic and community engagement.		