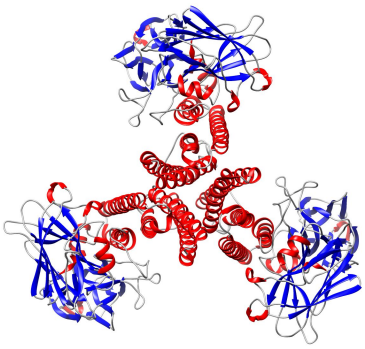
Chemistry 371: Advanced Biochemistry Laboratory

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 insecticidal crystal protein, Cry1Aa from *Bacillus thuringiensis* (Bt). Additionally, students will generate mutations within the toxin to increase the toxic effect against the yellow-fever mosquito, *Aedes aegypti*, with the ultimate goal of determining which amino acids, if any, are important for protein toxin to function.



Cry4Ba from *Bacillus thuringiensis*

Instructor and Course Resources

Instructor:	Prof. Matthew Rouhier ("Roo-yer")
Email:	rouhierm@kenyon.edu
Office:	208 Tomsich Hall
Class location:	Tomsich 203 (with pre-lab discussion in Tomsich 206)
Class time:	1:10-4:00 pm Tuesday
Materials:	Computer, calculator, and associated literature posted to Moodle
Websites:	*moodle.kenyon.edu* and *benchling.com*
Office Hours:	TBD

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

The American Society for Biochemistry and Molecular Biology has developed a list of learning goals as a guide for biochemistry and molecular biology programs. "Skills" is one of four foundational concepts developed by the Society and states "discovery requires objective measurement, quantitative analysis and clear communication." Based on their recommendations, this course will address: (1) the process of science, (2) the accessing, comprehending, and communication of science, and (3) the community of science. The course Moodle site addresses each of these overarching goals, the specific learning goals/objectives, and the associated assessments and activities.

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 – *Attendance at all laboratory sessions is mandatory.* If you know that you will miss a laboratory session for a scheduled event please contact the instructor immediately. I 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 a member of the Kenyon College Faculty, I am concerned about the well-being and development of our students and am available to discuss any concerns. However, I 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 – Laptops will be provided (or you may use your own) at each class to work up data. Please refrain from using it to email, play games, or any other activity unrelated to class. For your safety, cell phones are only to be used only 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 instructor reserves 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%
Sequence work	10%	Grant proposal: final	10%
Grant proposal: pre-proposal	15%	Toxicological 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).

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 Cry toxins (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 Cry toxins 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 Cry toxins as insecticides against *A. aegypti*. The format of the pre-proposal must follow these guidelines: 11 pt, Times New Roman font with 1 inch margins on all sides. 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 15th.

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 5th.

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 12th.

Full proposal (final submission) – The final, polished proposal is due by 5pm on April 26th.

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 should 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 during finals week on May 6th at 6:30 pm.

Supplemental office hours – Periodically throughout the semester, the instructor will hold office hours at Pierce Dining hall to discuss Cry toxins, class data, preparing for the next experiment, writing and communication scientifically, etc. Dates and times will be posted via Moodle and by email.

Schedule

Class	Team	Week: Experiment	Notebook[1]	Writing assignments (due 5pm Friday of lab week)
Jan 15	1	1: Intro, safety, check-in, make solutions		
Jan 22	1	2: Native Cry protein purification (day 1)	✓	Sequence work: basic information
Jan 29	1	3: Native Cry protein purification (day 2)	✓	Sequence work: sequence similarity
Feb 5	1	4: Native Cry protein purification (day 3)	✓	Sequence work: structural similarity
Feb 12	1	5: Determine [protein]	✓	Grant: pre-proposal
Feb 19	1	6: Determine protein purity	✓	
Feb 26	2	7: Mutant Cry protein (site-directed mutagenesis)	✓	
SPRING BREAK!!				
Mar 19	2	8: Mutant Cry purification (day 1)	✓	
Mar 26	2	9: Mutant Cry purification (day 2)	✓	
Apr 2	2	10: Mutant Cry purification (day 3)	✓	Grant: full proposal draft
Apr 9	2	11: Determine mutant [protein] and purity	✓	Grant: peer review
Apr 16	3	12: *A.aegypti* Toxicology	✓	
Apr 23	3	13: *A.aegypti* Toxicology		Grant: full proposal
Apr 30	3	14: *A.aegypti* Toxicology		
Final exam week		Monday, May 6th at 6:30 pm	Toxicological 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.

Learning Goals

The American Society for Biochemistry and Molecular Biology (ASBMB) developed a set of concept-driven learning objectives as guides for all Biochemistry and Molecular Biology programs. The learning goals of this course are based on those suggested by the ASBMB. CHEM 371 addresses the learning goals associated with the theme of "Skills" (there are four themes in all). I have included text from ASBMB for this particular theme and the components of the course associated with that learning goal. If you would like to learn more about ASBMB's concept-driven teaching, visit:
http://www.asbmb.org/education/teachingstrategies/

SKILLS: Discovery requires objective measurement, quantitative analysis and clear communication.

1. Process of science: The process of science combines creative ideas, experimentation and data analysis. Scientists develop a hypothesis and design and conduct appropriate experiments. Experimental results are analyzed and data interpreted using appropriate quantitative modeling and simulation tools.

Learning Goal/Objective	Learning Assessment	Details
Students should be able to accurately prepare and use appropriate volumes of reagents and perform the required experiments.	Lab work and Notebook entries	In the materials and procedure sections students will provide information on preparation of needed reagents.
When presented with an experimental observation, students should be able to develop a testable and falsifiable hypothesis.	Grant proposal and Toxicology work	Students will write a grant proposal based on primary literature that includes a hypothesis and experimental setup. Teams will also design a kinetics experiment to analyze the function of their mutant compared to the native enzyme.
When provided with a hypothesis, students should be able to identify the appropriate experimental observations and controllable variables.	Pre-lab presentations	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?
Students should be able to determine averages and standard deviations to relate the significance of experimentally obtained data.	Notebook entries	In the results section students will report averages and standard deviations where appropriate.
Students should be able to use appropriate equations to analyze experimental data and obtain parameters.	Notebook entries	In the results section students will show the use of appropriate equations and associated analysis.
Students should be able to use equations and models to predict outcomes of experiments.	Notebook entries	Students will be using enzyme kinetic models to predict the quality of the activity.

2. Accessing, comprehending and communicating science: Scientists access, assess and use available information and present data in appropriate contexts in a variety of ways at different levels.

Learning Goal/Objective	Learning Assessment	Details
Students should be able to identify, locate and use the primary literature	Grant proposal	Students will prepare a grant proposal that must include support from the primary literature.
Students should be able to use databases and bioinformatics tools.	Sequence work	Students will keep a notebook of data based on sequence alignments, structural similarities and enzyme function.
Students, when provided with appropriate background information, should be able to identify consistencies and inconsistencies.	Grant proposal	Students will use the data acquired during the semester to identify consistencies and inconsistencies with previously published data.
Students should be able to explain the big picture aspects of current challenges in the molecular life sciences.	Grant proposal and Toxicology presentation	The grant proposal will a section on the broader impact of this work, while the enzyme kinetics presentation will include a discussion on how the novel data adds to the current body of data.
Students should be able to use visual and verbal tools to explain concepts and data.	Toxicology presentation	Students will give a final presentation sharing their data with the class.
Students should be able to translate science into everyday examples.	Grant pre-proposal	The pre-proposal will be written so that a variety of scientists can understand the significance of this work.

3. Community of practice: Science is interdisciplinary and relies on collaboration, effective teamwork, safety and ethical practices.

Learning Goal/Objective	Learning Assessment	Details
Students should explain the importance of and keep an accurate laboratory notebook.	Notebook entries	Students will be using Benchling to keep a laboratory notebook.
Students should be able to access and interpret safety information and conduct lab work safely and ethically.	Lab work, Pre-lab presentation, and Notebooks	Students will undergo lab safety training on the first day and will note particular hazards in their notebooks associated with that week's experiment.
Students should be able to give and take directions to be an effective team member.	Lab work	Students will be working in teams to accomplish experimental tasks. Their ability to work as an effective team will be assessed periodically throughout the semester.