# **Course Description**

BCMB 406B is a project-based course that will build on research skills you have developed in previous lab courses. Unlike other lab courses that consist of several distinct labs, 406B has three labs that build on each other to create a continuous research project from start to finish. The overall aim of 406B is to create and characterize a mutant carbohydrate binding module (CBM). CBMs are accessory modules of glycoside hydrolases (GH) which are enzymes that hydrolyse the glycosidic bond between carbohydrates. As the name suggests, the CBM targets the enzyme to its substrate by binding to carbohydrates.

In lab 1, you will learn the principles of primer design and use a variety of web-based tools to design and evaluate a set of primers for site directed mutagenesis.

At the start of Lab 2, you will use molecular biology software to plan and predict the results of individual steps in the site-directed mutagenesis of a CBM gene. You will then use inverse PCR to generate the site directed mutant, using restriction digests and DNA sequencing to confirm the presence of the desired mutation. Once the correct mutation has been confirmed, you will move the newly created mutant CBM construct into an expression host.

Lab 3 focuses on the purification and characterization of the mutant protein. Initially, you will induce expression of the mutant CBM protein and purify the protein using Immobilized Metal Affinity Chromatography (IMAC). Once purified, you will assess the mutant CBM's ability to bind carbohydrate using two techniques that will allow you to compare the function of the mutant CBM to that of wild type. Finally, you will attempt to crystallize the mutant protein and use modelling software to compare and contrast the structures of the mutant and wild type CBMs.

In this course, emphasis is placed on experimental design, data analysis and problem solving with the intention of developing your ability to work independently in the lab.

# **Intended Learning Objectives for 406B**

Upon completion of 406B you will be able to:

- Describe the theory and principles of primer design, site-directed mutagenesis and protein expression, purification and characterization
- Develop proficiency in practical skills and *in silico* techniques used for primer design, sitedirected mutagenesis and protein expression, purification and characterization
- Solve typical calculations used in biochemistry and microbiology experiments
- Evaluate experimental controls
- Generate a written record of data in a lab journal
- Evaluate data generated and summarize findings in written lab reports
- Compare and contrast data generated in the laboratory with that of relevant published research articles

Week	Dates	Lab	Day 1 (5 hours)	Day 2 (2 hours)	Due Dates					
1	Jan 11-15	Lab 1: Primer Design	Primer design and evaluation using web-based tools							
2	Jan 18-22	Lab 2: Site Directed Mutagenesis of CBM Proteins	In silico cloning		Day 2: Lab 1 Report					
3	Jan 25-29		Inverse PCR, agarose gels, DpnI digestion, electrocompetent cells	Electroporation						
4	Feb 1-5		Plasmid purification, restriction digests, agarose gels	Plasmid preps, DNA quantification, sequencing	Electroporation control assign't					
5	Feb 8-12	Lab 3: Purification and Characterization of a Mutant CBM	Lab 2 - Chemical transformation Lab 3 - Media prep	Lab 2 - Count plates Lab 3 - Starter culture						
6	Feb 15-19	Ν	lo Labs – Reading Break	ζ						
7	Feb 22-26	Lab 3: Purification and Characterization of a Mutant CBM	Inoculate/induce large culture, prep solutions, WT macroarray	Pellet cells, macroarray development	Day 1: Lab 2 Report					
8	Mar 1-5		Chemical lysis of cells, IMAC: nickel column chromatography	Identify protein- containing fractions						
9	Mar 8-12		SDS-PAGE of fractions	Dry gels, pool fractions, dialysis	Midterm exam (Labs 1 and 2) Thurs. Mar. 11 7-9 pm					
10	Mar 15-19		Macroarray (mut vs WT), A <sub>280</sub> , Affinity gels, Lysozyme crystallization	Macroarray development, Dry gels						
11	Mar 22-26		Gel RMD measurements Lysozyme crystallization PyMol Help	Visualize crystals						
12	Mar 29 - Apr 1				Day 1: Lab 3 Report					
TBA – Lab 3 exam during regular exam schedule										

# **Evaluation and Assessment**

# Percentage Breakdown for the Course:

Exams	40 %
Lab Reports	30 %
Practical Assessment	20 %
Laboratory Journal	10 %

#### UVic Grading Scheme

<b>A</b> +	90 -100	B⁺	77 - 79	C⁺	65 - 69	F	<	50
Α	85 - 89	В	73 - 76	С	60 - 64	N **	<	50
A-	80 - 84	B-	70 - 72	D	50 - 59			

#### \*\* N grades

Students who have completed the following elements will be considered to have completed the course and will be assigned a final grade:

(list all components to be considered, e.g. assignments, midterms, final exam, etc.)

Failure to complete one or more of these elements will result in a grade of "N" regardless of the cumulative percentage on other elements of the course. An N is a failing grade, and it factors into a student's GPA as 0. The maximum percentage that can accompany an N on a student's transcript is 49.

#### DEPARTMENT INFORMATION AND POLICIES

- The Department of Biochemistry and Microbiology upholds and enforces the University's policies on academic integrity. These policies are described in the current University Calendar. All students are advised to read this section.
- 2. Cell phones, computers, and other electronic devices must be turned off at all times during live class sessions unless being used for the purpose of connecting and engaging with the class.
- 3. No recordings of live lectures are permitted without permission of the instructor. Many online courses will be recorded by the instructor for accessibility for students unable to attend. If you do not wish to be recorded, contact your instructor to determine if alternative arrangements can be made.
- 4. Course materials, such as notes, problem sheets, quizzes, examinations, example sheets, or review sheets, may not be redistributed without the explicit written permission of the instructor.
- 5. Students are expected to be available for all exams. Instructors may grant deferrals for midterm examinations for illness, accident, or family affliction. Although students do not require documentation, students must contact their instructor and BCMB office (<u>biocmicr@uvic.ca</u>) with the reason for their absence within 48 hours after the midterm exam. The Department will keep a record of the absences. It is the responsibility of the student to ensure all required components are complete, and to arrange deferred exams/assignments with the instructor, which normally should occur within one week of the original exam date.
- 6. The Department of Biochemistry and Microbiology considers it a breach of academic integrity for a student taking a deferred examination to discuss the exam with classmates. Similarly, students who reveal the contents of an examination to students taking an examination are considered to be in violation of the University of Victoria policy on academic integrity (see current University Calendar). Students must abide by

UVic academic regulations and observe standards of scholarly integrity (no plagiarism or cheating). Online exams must be taken individually and not with a friend, classmate, or group, nor can you access notes, course materials, the internet, or other resources without the permission of the instructor. You are prohibited from sharing any information about the exam with others. Use of unauthorized electronic devices and accessing the internet and class material during exams is prohibited unless permission is granted by the instructor. Instructors may use Browser Lockdown Software to block access during classes and exams.

- 7. Deferral of a final exam must be requested with an Academic Concession form and submitted directly to Undergraduate Records. Deferred final exams for fall term courses will be arranged by the instructor. Deferred final exams or spring term courses will be arranged through Undergraduate Records and must be written before the end of the summer term as stipulated in the University Calendar.
- 8. Requests for review/remark of a midterm exam must be made within one week of the exam being returned.
- 9. The instructor reserves the right to use plagiarism detection software or other platforms to assess the integrity of student work.
- 10. Supplemental exams or assignments will not be offered to students wishing to upgrade their final mark.
- 11. Anonymous participation in online classes is not permitted without permission of the instructor.

### Important note about COVID-related stress

The current pandemic is placing added stressors- financial, mental, and physical- on everyone. Your wellbeing is of foremost importance. If you are experiencing difficulties coping, the University has resources to help. Reach out to Counselling Services, the Centre for Academic Communication, or Learning Assistance Program for assistance.

### **Centre for Accessible Learning**

Students with diverse learning styles and needs are welcome in this course. In particular, if you have a disability/health consideration that may require accommodations, approach the Centre for Accessible Learning (CAL) as soon as possible in order to assess your specific needs. <u>https://www.uvic.ca/services/cal/index.php</u>

# **Course Experience Survey (CES)**

We value your feedback on this course. Towards the end of term you will have the opportunity to complete a confidential course experience survey (CES) regarding your learning experience. The survey is vital to providing feedback to us regarding the course and our teaching, as well as to help the department improve the overall program for students in the future. When it is time for you to complete the survey, you will receive an email inviting you to do so. If you do not receive an email invitation, you can go directly to your <u>CES</u> <u>dashboard</u>. You will need to use your UVic NetLink ID to access the survey, which can be done on your laptop, tablet or mobile device. We will remind you nearer the time but please be thinking about this important activity.