Name and Title: Warren Lee, Assistant Professor
Department: Biochemistry

TITLE OF RESEARCH PROJECT: LDL Transcytosis by Coronary Endothelial Cells and the Initiation of Atherosclerosis

Number of 299Y Spots: 1  Number of 399Y Spots: 1

OBJECTIVES AND METHODOLOGY:
The accumulation of LDL under the coronary arterial endothelium is the first step in the pathogenesis of atherosclerosis, one of the commonest causes of death in Canada. How LDL exits the vascular lumen and crosses the endothelial monolayer is very poorly understood, in large part due to technical limitations in studying endothelial permeability. Our lab has devised novel assays to measure how LDL crosses the endothelium and is currently delineating the molecular mechanisms. Ultimately this work may lead to novel approaches to both the prevention and treatment of atherosclerosis. The methodology used includes high-resolution live cell imaging as well as traditional biochemical and molecular biology techniques. For further details, please consult the lab website (warrenleelab.com)

DESCRIPTION OF STUDENT PARTICIPATION:
Experience with cell culture is preferred, and enthusiasm and industriousness are essential. Under appropriate supervision, the student will perform primary cell culture, live cell imaging, immunoblotting and immunofluorescence and will be taught analytical and presentation skills.

MARKING SCHEME (assignments with weight and due date):

2-page Interim report 25%, due Nov. 15th.
Lab journal 35% - due at end of term
Attendance and participation in lab meetings 15%
Final oral presentation at lab meeting on the project 25% - at end of term
Name and Title: Greg Fairn, Associate Professor  
Department: Biochemistry  

TITLE OF RESEARCH PROJECT: Development of Cellular and Genetic models to investigate Lenz-Majewski Syndrome  
Number of 299Y Spots: 1  
Number of 399Y Spots: 1  

OBJECTIVES AND METHODOLOGY:  
Background: Lenz-Majewski is a syndrome of intellectual disability and multiple anomalies including skin and bone defects. The etiology of this syndrome is gain-of-function mutations in the phosphatidylserine synthase 1 (PTDSS1) one of the two enzymes responsible for the synthesis of the aminophospholipid phosphatidylserine (PtdSer). Currently, it is unclear why excess PtdSer is detrimental.  

The objective of this project is to develop human and yeast models of this syndrome for cellular, biochemical and genetic analysis.  

Methodology: To increase phosphatidylserine synthase activity in yeast, cells will be transformed with a plasmid encoding a galactose-inducible yeast PtdSer synthase or with the human gain-of-function variant. The students will determine the extent of the PtdSer increase and its impact on cell growth and vesicular transport pathways (i.e. secretion, endocytosis). Once ideal conditions are identified the strains of interest will be used for genetic screens.  

Standard mammalian cell culture will be used to cultivate mammalian cells. HeLa and HEK293 cells stably expressing the tetracycline repressor will be used to integrate the wild-type and mutant PTDSS1. Fluorescence microscopy will be used to monitor the distribution of phosphatidylserine and other phospholipids. The transit of cargo (collagen, ssHRP and VSVG\textsuperscript{1}) through the secretory pathway will be monitored by using a combination of microscopy, Western blotting and chemiluminescence.  

DESCRIPTION OF STUDENT PARTICIPATION:  
The ROP299/399 students will be expected to read appropriate background literature with guidance from the supervisor. Experimental planning and training in various techniques will be provided by the senior graduate students, technical staff or Dr. Fairn with the student carrying out procedures on a semi-independent basis. As the project progresses, the students will participate in planning experiments. The students will be expected to keep accurate and complete records of their experimental work, and the notebook will regularly be viewed by the supervisor who will make suggestions for improvement of record keeping. The student will be expected to meet with the supervisor on a weekly basis and attend lab meetings.
Techniques include:

1) Yeast cell culture and DNA transformation
2) PCR, site-directed mutagenesis, and sub-cloning
3) Generation of tetracycline inducible mammalian cell lines using Flp-In TRex cells.
4) Cell growth assays and fluorescence microscopy
5) Western blotting
6) Thin-layer chromatography

MARKING SCHEME (assignments with weight and due date):
1. Project Proposal (2 pages plus figures and references, due within 3-4 weeks after starting) 20%
2. Oral Project Presentation (20 min at the mid-point) 10%
3. Lab performance, work ethic, lab notes, participation. 20% with 10% assigned at mid-point
4. Final Oral Presentation (20 min plus ability to answer questions) 20% during final week
5. Final Lab Report (8-10 pages plus figures and references) 30% during final week
Name and Title: Walid Houry, Professor
Department: Biochemistry

TITLE OF RESEARCH PROJECT: The Development of Novel Antibiotics

Number of 299Y Spots: 1 Number of 399Y Spots: 1

OBJECTIVES AND METHODOLOGY:
In recent years, there has been an alarming trend of increased bacterial infections caused by strains resistant to most known drugs. As a result, diseases that were thought to be controlled by currently available antibiotics are re-emerging not only in developing countries but also in industrialized nations, especially in clinical settings such as hospitals. Therefore, there is an urgent need for the development of new types of antibiotics that can be used to effectively treat multidrug resistant bacteria. In this project, we propose to screen and develop a novel class of antibacterial drugs that can activate highly-conserved, tightly-regulated, self-compartmentalizing cylindrical proteases in bacterial cells. One such protease is ClpP. On its own, ClpP can only degrade small peptides and not folded proteins. The binding of unfoldase chaperones to ClpP is required for the degradation of native proteins. ClpP has recently been validated as a novel molecular target for antibacterial drug development. We aim to develop and identify novel compounds that allow ClpP and other such cylindrical proteases to indiscriminately degrade folded proteins eventually causing bacterial cell death. The efficacy of the compounds will be tested using model infectious bacterial systems. These compounds will define a new class of antibiotics, namely activators of self-compartmentalizing proteases, which we will refer to as ACPs. Students in BCH299 will work on characterizing the biochemical consequences of the binding of ACPs to the cylindrical proteases using purified proteins and pertinent biophysical methods. Students will not be involved in working with any pathogenic bacteria.

DESCRIPTION OF STUDENT PARTICIPATION:
The student will be involved in cloning different genes using standard molecular biology techniques, in purifying proteins by mainly using chromatography techniques and in carrying out various biochemical assays with the purified proteins. Students will work closely with a senior graduate student or postdoctoral fellow.

MARKING SCHEME (assignments with weight and due date):
5% two-page report due mid-November
10% 20-minute presentation in mid-November
10% work in the laboratory in mid-November
25% 15-page report at end of term in April
15% 30-min presentation at end of term in April
35% work in the laboratory at end of term in April
RESEARCH OPPORTUNITY PROGRAM
299Y/399Y PROJECT DESCRIPTIONS 2019-2020
FALL/WINTER

Name and Title: Dr. G. Angus McQuibban
Department: Biochemistry

TITLE OF RESEARCH PROJECT: Identifying Small Molecule Therapeutics for Parkinson’s Disease

Number of 299Y Spots: 2 Number of 399Y Spots: 1

OBJECTIVES AND METHODOLOGY:
We conduct ongoing screens of small molecules to identify potential therapeutics to combat neurodegenerative diseases like Parkinson’s disease. My lab has had a long-standing interest in understanding mitochondrial function in basic cell biology, and more recently, in the mechanisms of human disease. Recently, it has become established that mitochondrial function is key to prevent the death of neurons, directly linking mitochondria to neurodegeneration. We use a combination of cell biology and biochemistry to understand how the molecules identified in our screens alter mitochondrial biology.

DESCRIPTION OF STUDENT PARTICIPATION:
Student(s) will be engaged in the project under the direct supervision of the Research Associate conducting the screens. After initial training, it is expected that students will conduct their experiments independently. Techniques used will include Western blotting, cell culture, and Drosophila studies. Participation in lab meetings and journal clubs is also expected.

MARKING SCHEME (assignments with weight and due date):

Research proposal at the beginning of the course (10%).
2 Presentations in group meetings at the beginning and end of the course
of 1 presentation in group meeting and 1 poster at the Undergraduate Research Forum at Hart House (30%).
Lab performance (30%).
End term report (30%).
Name and Title: Trevor Moraes, Associate Professor
Department: Biochemistry

TITLE OF RESEARCH PROJECT: Structural and Functional Examination of Membrane Proteins from Pathogenic Bacteria

Number of 299Y Spots: 1 Number of 399Y Spots: 1

OBJECTIVES AND METHODOLOGY:
Disease-causing bacteria rely on the acquisition of a diverse set of nutrients from their host environment to engage in successful pathogenesis. Overcoming nutritional immunity imposed by the host is essential and Gram-negative pathogens such as Neisseria, Pseudomonas, Enterohemorrhagic Escherichia coli (EHEC) O157:H7, Salmonella typhimurium and many others are reliant on specific transport machineries in the outer membrane (Pogoutse et al. Crit Rev Biochem Mol Biol. 2017) and inner membrane to overcome this challenge (Sit el al. PLoS Pathogens 2015).

The goal of this research is to analyze the interactions between these proteins and the nutrients that they steal from their hosts. The project will involve purifying proteins and examining their interactions using biophysical methods including; X-ray protein crystallography to determine the 3D structure of proteins; biolayer interferometry (BLI), or microscale thermophoresis (MST) that can be used to measure the affinities between proteins and small molecules; finally the students will perform bacterial growth assays with difference versions of the transporters to test how mutations affect bacterial fitness and the ability for the bacteria to infect a host.

DESCRIPTION OF STUDENT PARTICIPATION:
Students will be paired with a research technician and a graduate student who will train them on each aspect of the project including creating site directed mutants of proteins involved in Ion translocation and then expressing, purifying and characterizing these mutants. The students will participate in the cloning (PCR amplifications, ligations and transformations) and expression of the derivative proteins. Utilizing affinity, ion exchange and size exclusion chromatography on an FPLC the students will purify these proteins. Purified protein will be analyzed to determine binding affinities compared to the wild-type protein (methods include: BLI, MST). Structures of interesting mutant proteins will be determined using protein crystallography. A weekly meeting between the ROP student, research technician and graduate student will be used to monitor progress and discuss the upcoming research plan.

MARKING SCHEME (assignments with weight and due date):
Students will be evaluated on presentations and participation in lab meetings, lab notebook and lab performance (40% -lab members will help in the evaluation of; Work ethic , Understanding of their biological system, Lab
skill/ability to learn new techniques, Independence, Lab notebook, Contribution to lab meetings, Presentations, Productivity), a research proposal (20%, 1pg “summary page” ~ due 3-4 weeks after starting the project followed by a 5pg NSERC style research proposal @ 4-8 weeks), a midterm report on results (15%- detailed methods and results due midway through term), and an end of term report (25% due on the last day of classes – Journal of Biological Chemistry style report ).