Course Documents

SYLLABUS

BioSci 1005: Introduction to Biochemistry Lab
Fall Term 2018

General Information
Lab Sections:  Wednesday, 1:00 - 4:50 PM
Location:  A146-A148 Langley Hall

INSTRUCTORS: Dr. Andrew VanDemark
A360 Langley Hall
412-628-0110
andyv@pitt.edu

Teaching Assistant: Matt Googins
A306 Langley Hall
mrg81@pitt.edu

Special Assistant: Dr. Krista Freeman
kgf10@pitt.edu

Prerequisites & Co-requisites
The prerequisite for the course is BIOSC 0060 Foundations of Biology 2 Laboratory, and the co-requisite is BIOSC 1000 Biochemistry. Having taken and passed General Chemistry I will be very helpful, but not required.

Course Objectives and Summary
The objective of this course is for you to use a variety of biochemical and biophysical techniques to help establish a function for a novel viral protein of unknown function. Our novel viral genes come from mycobacteriophage, viruses that infect mycobacteria including those that cause tuberculosis and leprosy. Through our connection with the PhageHunters and Sea-phages projects, we have access to an incredible collection of mycobacteriophage genome sequences, currently numbering 1,435 as of Dec 12th, 2017. The genomes house a staggering 220,363 individual protein sequences, which can be clustered into 20,538 unique families (or Phamilies in the Phage world). Nearly 70% of these Phamilies have unknown function or structure. That means that there are literally tens of thousands of different proteins within this collection that are complete unknowns. These sequences represent a unique collection of sequence space which has not been sampled by modern biology and could contain new protein folds, protein capable of performing new types of chemistry, or new types of anti-CRISPR-like systems that control host-phage interactions.

The tricky part here is that it is very hard to study a protein with a completely unknown structure or function. Simply too many unknowns to design useful experiments. Therefore, we have devised a strategy to select for sequences within our pool that are more likely to purify and crystallize. We have also initiated some very straightforward and basic biochemical assays designed to shed a little light on
the basic function(s) of the protein. Our overall hypothesis is that we can use these initial biochemical insights in combination with structural information via x-ray crystallography to make detailed predictions about the function of proteins within this collection.

We will assign you two of these genes selected from our pool of the most promising candidates for study. You will generate a recombinant expression system to making a fluorescently tagged version of this protein which you will express and purify. You will design experiments to test the oligomeric state and structural integrity of your protein. You will begin to learn about the function of your protein by seeing where it localizes within a bacterial cell and identifying bacterial proteins that it interacts with. Lastly, you will make some initial attempts at crystallizing this protein. You will wrap up all of these experiments into a single comprehensive report at the end of the year which details all that you have learned about your gene and during which you will predict the function of the protein.

This course is an upper division biochemistry laboratory for General Molecular Biology Majors, at an intermediate level. While the term “intermediate” level may seem disarming, you will find this is an exciting and hands-on learning environment that emphasizes critical and creative thinking as well as the ability to evaluate your data in an evenhanded way. This is a truly inquiry based class, and we honestly do not know what the answers are. You will be the first person on the planet to have ever studied the proteins you select, and everything you learn will therefore be new and in its own way groundbreaking. Along the way, you will learn how to troubleshoot experiments, fill in gaps within protocols, enhance your skills at lab math, and to start independently evaluate data.

**Course Requirements**

*Format and Time Expectations:* The lab will meet for 4 hours per week. The class format for each week will vary a bit depending on the time constraints dictated by the experiments. You should expect to spend as much time outside of class preparing for the lab, doing homework, reading background material, and preparing presentations.

*It is crucial that you have read your experimental procedure and completed any prelab prior to class. Otherwise, you will likely not be able to complete the experiment within the allotted time.*

We will often have a short lecture at the beginning/middle of the lab period to make sure that everyone has prepared for class and to give you an extra opportunity to ask questions about that week’s experiment, overall course design, or anything else that you would like to learn about. Many class periods have assignments which will be completed within the class period. While some portions of the labs will be performed in teams of two, your assignments are to be completed individually.

*Text:* There is no textbook for the course. Your Xtalhunters course manual will be your primary course material, which will be supplemented by a variety of outside materials as appropriate including primary research papers (peer-reviewed publications), reviews, and third party research protocols.

*Computers*

You will need to use computers (available in the lab or the HHMI Computing Labs), to look up web-based material to perform your lab work, especially within the first couple of weeks. You will perform bioinformatics searches, make DNA sequence maps, and visualize these using programs that are web-based or are freely available and thus you can also perform all of this analysis on any computer that you
have access to. Additionally, you will need access to your Pitt account to receive class information and to turn in many of your assignments (see below).

If you are having any issues with computer access or functionality, please let me know immediately so that we can find a solution.

**Communication & Distribution of Information**

Nearly all course material will be distributed to you via CourseWeb. All communication will occur through your pitt.edu email account, thus you are required to maintain a functional “pitt.edu” account.

**Turning in your work**

All documents (including reports, in-class assignments, quizzes, final report, etc) will be turned in through CourseWeb. Please include your last name or initials, followed by the assignment name. This is a valuable habit for now and your future because it is a) courteous and b) insurance that your document is properly credited to you.

VanDemark_Cloning_Report.docx is an example of a good filename

**Work Load**

**Pre-lab assignment**
A selected number of weeks have a pre-lab assignment. You will be given that assignment during the week before it is due and it will be turned in at the beginning of class. The purpose of this is to ensure that you are properly prepared for the experiments you will perform in the lab, or that you are prepared for the in-class assignment that particular week.

**Notebook**
- We will maintain a database (either hardcopy or electronic) for you to deposit your lab results from each experiment.
- Include your actions, your observations, your rationale, your thoughts, and your interpretations in the record. Do not include printed protocols that we gave you, although it may be wise to outline these protocols in your notebook.
- Most importantly, include your data!! Marked up and Annotated so that anyone else coming into this experiment fresh could interpret it.

**Policy on the Export or Import of Lab Materials**

We do not permit any student to take any teaching lab samples, reagents or other materials to their research lab or back from their research lab to the Langley Teaching Lab. This policy is directed to biological reagents and samples, and it does not include electronic or paper documents. When students take experiments back to another lab space, or carry supplies from their lab to the teaching labs, we cannot control the experiment or personal safety, which it is our responsibility to do.
**DISABILITIES RESOURCE SERVICES**

If you have a disability for which you are or may be requesting an accommodation, you are encouraged to contact both your instructor and Disability Resources and Services, 216 William Pitt Union, [412-648-7890/412-383-7355(TTY)] as early as possible in the term. DRS will verify your disability and determine reasonable accommodations for this course.

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**ACADEMIC INTEGRITY POLICY**

Cheating/plagiarism will not be tolerated. Students suspected of violating the University of Pittsburgh Policy on Academic Integrity, noted below (**), will be required to participate in the outlined procedural process as initiated by the instructor. A minimum sanction of a zero score for a quiz, exam, or paper will be imposed.

** The integrity of the academic process requires fair and impartial evaluation on the part of faculty and honest academic conduct on the part of students. To this end, students are expected to conduct themselves at a high level of responsibility in the fulfillment of the course of their study. It is the corresponding responsibility of faculty to make clear to students those standards by which students will be evaluated, and the resources permissible for use by students during the course of their study and evaluation. The educational process is perceived as a joint faculty-student enterprise which will perforce involve professional judgment by faculty and may involve—without penalty—reasoned exception by students to the data or views offered by faculty. Senate Committee on Tenure and Academic Freedom, February 1974

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**Email Communication Policy**

Each student is issued a University email address (username@pitt.edu) upon admittance. This email address may be used by the University for Official Communication with students. Students are expected to read e-mail sent to this account on a regular basis. Failure to read and react to University communications in a timely manner does not absolve the student from knowing and complying with the content of the communications. The University provides an email forwarding service that allows students to read their email via other service providers (e.g., Hotmail, AOL, Yahoo). Students that choose to forward their e-mail from their pitt.edu address to another address do so at their own risk. If e-mail is lost as a result of forwarding, it does not absolve the student from responding to official communications sent to their University e-mail address. To forward e-mail sent to your University account, go to [http://accounts.pitt.edu/login/toyouraccount.click](http://accounts.pitt.edu/login/toyouraccount.click) on Edit Forwarding Addresses, and follow the instructions on the page. Be sure to log out of your account when you have finished. (For the full E-mail Communication Policy, go to [www.bc.pitt.edu/policies/policy09/09-01.html](http://www.bc.pitt.edu/policies/policy09/09-01.html).)

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**Office Hours**

Office hours for either the Instructor or the Teaching Assistant can be Monday-Friday between 9:00am and 5pm, but because of our uneven schedules, Office Hours must be arranged by appointment. All relevant contact information (Office location, phone number, and email addresses) can be found in the first page of syllabus.

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**GRADING**
Lab experimental techniques and performance, lab data, homework, quizzes, pre-tests and a final report will all be components of the final grade. Within the laboratory, we expect you to:

- Be prepared in advance by reading relevant protocols or background materials, as suggested
- Show effort and improvement in your lab techniques so you improve your skills of experimental analysis, experimental design and experimental interpretation
- Demonstrate safe and responsible laboratory practices
- Work well with others, most importantly, with your lab partner, and show proper laboratory etiquette
- Be prepared to interact verbally and respond to questions during lecture and lab

Attendance is mandatory for all lab and lecture sessions. All absences must be excused directly by the instructors prior to the class meeting. Email is the best method. All absences must be justified, and we reserve the right to require documentation. When students are unable to attend, we will try to make the lab material and experiments available at alternative times, but this may not always be feasible.

**Point Distribution**

The total points possible in this class will be distributed approximately as follows:

<table>
<thead>
<tr>
<th>Grade assignments (take home and in-class combined)</th>
<th>630 points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Poster Presentation</td>
<td>200 points</td>
</tr>
<tr>
<td>Lab Citizenship</td>
<td>50 points</td>
</tr>
<tr>
<td>Participation</td>
<td>120 points</td>
</tr>
</tbody>
</table>

**Total Points**

1000 points

Currently, the point’s breakdown for the course is:

<table>
<thead>
<tr>
<th>Date</th>
<th>Graded Items</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>In class</td>
<td>29-Aug</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• CURE intro survey</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>• Lab Math</td>
<td>0</td>
</tr>
<tr>
<td>In class</td>
<td>5-Sept</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Protein Sequence Analysis for both GeneX and Ruby-GeneX fusion (Bioinformatics worksheet)</td>
<td>50</td>
</tr>
<tr>
<td>Turned in on</td>
<td>11-Sept</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Pymol Worksheet</td>
<td>25</td>
</tr>
<tr>
<td>Turned in on</td>
<td>3-Oct</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Protein Purification Report</td>
<td>150</td>
</tr>
<tr>
<td>In class</td>
<td>3-Oct &amp; 10-Oct</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Present Overall hypothesis of GeneX function (two parts split across two classes)</td>
<td>75</td>
</tr>
<tr>
<td>Turned in on</td>
<td>17-Oct</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Limited Proteolysis Assignment</td>
<td>100</td>
</tr>
</tbody>
</table>
### Lab Citizenship

**Turned in on 7-Nov**
- EMSA Report: 40

**Turned in on 14-Nov**
- Assignment on cytotoxicity: 50

**Turned in on 21-Nov**
- BioID pull-down Report: 100

**In-class 28-Nov**
- Localization Short Report: 30
- Cure Post Survey: 200

**In class 5-Dec**
- Final Presentation: 200

### Lab Citizenship
- Participation: 50

**Total**: 1000

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**Lab Safety**

### General Safety

- **Eye Wash Stations, Shower, First Aid Kits**: know their locations.
- **Hazardous Materials** (acids, bases, toxins, etc.): know where they are and after use, put them back where they came from.
- **Hazardous Waste Disposals**: use them appropriately. Disposal is expensive.
- **Glass Waste Bins**: glass only, not gloves, plastic, tissues, etc.
- **Biohazard Waste Bins**: experimental plates only
- **Ethidium Waste Bin**: Gels only, not gloves and paper towels
- **Gloves**: always wear gloves in the lab in the lab.
- Exception: Remove gloves when using computers.
- **Wash Hands Always**: whenever you leave the lab.
- **Wear appropriate clothing**: Labcoats are available.
- Goggles/Eyeglasses are recommended and available.
- Contact wearers must wear eye protection when handling hazardous materials.
- No food or drink in the labs.
- No cell phones use in the lab (includes texting).
In the event of fire, evacuate the lab. Never try to fight the fire – make sure your lab mate makes it out.

Be a conscientious citizen – inform lab personnel of any concerns or potential hazards, such as spills of unknown origin and do your part to keep the lab clean & organized.

Never use equipment without proper instruction: particularly centrifuges.

Instrument Safety

Some of the instrument (or machines) in the lab can present a danger to people if the equipment is not used properly. To properly use it, you are required to read the following information and become properly trained where necessary, which is determined by your instructor and lab personnel.

All the instruments in the lab are precision tools that require proper calibrations. Proper usage insures these calibrations will be maintained and that student experiments can be successful.

All the instruments in the lab are expensive and time consuming to maintain. Proper usage reduces costs and allows us to provide the most bang for your buck.

Centrifuge Safety

There are two general classes of centrifuges in use in the Langley Teaching Labs:

- Table Top centrifuges:
  - for quick spins of 2 ml or less which do not exceed 17,000 rpm
  - Eppendorf 5418, one Hermle LabNet

- Sorvall Centrifuges:
  - referred to as “Floor model” centrifuges that look like washing machines
  - handle large volume samples (greater than 2 ml to 250 ml)
  - exceed 20,000 rpm
  - potentially dangerous & very expensive - usage requires training, (see below)

Table Top Centrifuges: What you must do

- Balance Tubes: When loading Eppendorf tubes, they must be distributed evenly in the rotor. For instance, in an 18-position rotor, if you use position #1, then always be sure a tube of approximately the same volume is in position #9. Three equal weight samples can be distributed in positions #1, #6, and #13. Usually an equivalent volume is sufficient for balance.

- Keep it Clean: If a tube leaks or breaks, or if any solution wets the rotor or the inside, attempt to clean it or notify lab personnel. If you know the liquid spilled is not dangerous, attempt to clean it up and inform lab personnel.

Sorvalls Centrifuges: What you must do

- You must be trained in use the Sorvalls. Do not use a Sorvall until you have had training to do so.

- You must fasten the lid of the rotor completely. There is no wiggle room for this. Not fastening a rotor lid destroys the rotor.

- Carefully balance your centrifuge bottles with their lids on the “trip balance”, for which you will be trained.
- **Never overfill a centrifuge bottle.** At speed the liquid will assume a vertical position that readily leaks out of overfilled containers. A 500 ml centrifuge bottle can only safely hold 250 ml.

- **Always stay with the Sorvall as it goes to speed.** If there is a problem such as an imbalance, they can be detected early and the machine **MUST** be shut off immediately to minimize the danger and the damage.

- **Keep it Clean:** If a tube leaks or breaks, or if any solution wets the rotor or the inside, attempt to clean it or notify lab personnel. If you know the liquid spilled is not dangerous, attempt to clean it up and inform lab personnel.

**Centrifuge Failure**

Over time, centrifuge rotors fatigue as a result of the powerful mechanical forces to which they are subjected. These mechanical forces are exacerbated by improper usage; a) unbalanced tubes, and b) chemical spills that are not cleaned out of rotor contribute to weakening of its metal. Modern centrifuges are designed to contain the rotor in the event of a failure to keep the room occupants safe.

The most common causes of centrifuge malfunctions include:

- Failure to place the lid on the rotor. (Sorvall only)
- Improper balancing of centrifuge tubes.
- Failure to properly secure the rotor to the drive.
- Overloading the rotor’s maximum mass.
- Utilization of centrifuge tubes that are not rated for the correct speed.

**If a centrifuge malfunctions**

- Turn off the centrifuge immediately & do not attempt to open the lid.
- Inform your instructor or lab personnel.