PUBS 2017

Biophysics 205A: Physical Underpinnings of Biological Systems

Fall 2017 Syllabus

Location: Genentech Hall Teaching Lab - Room 227

Course Days/Hours: Monday, Tuesday, Wednesday 1pm-5pm

Final presentations: October 26, 3 pm

Instructor: Martin Kampmann [1]

PUBS fellow / Course Coordinator: Robert Newberry [2]

Co-Instructors: Sy Redding, Seemay Chou, Hani Goodarzi

TAs: Taylor Arhar, Alison Maxwell, Daniel Schwarz, Douglas Wassarman, Taia Wu


Teams:

The alpha-team: Yessica, Jenna, Nish, Adam (TA: Alison) - compound: MG132

Rub-a-dub-dub 4 geeks in PUBS: Andrew, Taylor, Jean, Kyle (TA: Doug) - compound: tunicamycin

Yeast mode: Erik, Matt, Gracie, Lakshmi (TA: Taia) - compound: menadione

Buds: Jared, Snow, Christa (TA: Dan) - compound: spermidine

Aged Neuron Merge: Daniel, Maureen, George (TA: Taylor) - compound: geldanamycin

Course Credit: 4 units

Course Format: 12 hours of lab per week
Prerequisites: All incoming first year iPQB graduate students are required to enroll in this course. Although TAs, instructors, and your fellow students will be happy to help out, it is important to be familiar with basic scripting and the principles of python PRIOR to starting the class. This will be covered in bootcamp.

Grading: Letter grade

Textbook: None. Lab protocols and course materials will be available in class or online

Background

Current initiatives in biomedical research seek detailed understanding of the complex molecular basis for both normal physiology and disease pathology with the aim of developing targeted therapies uniquely suited to individual patients. This effort toward precision medicine involves a variety of discovery- and hypothesis-driven studies of the molecular, cellular, genetic, systems, and environmental contributions to biology and disease. Comprehensive, unbiased explorations of the cell and its components are key to this effort, as they illuminate the specific lesions underlying disease processes. Targeted interventions in cancer represent the greatest success story of precision medicine to date; many patients can be screened for disease-driving mutations that can be treated with specific molecules. Nevertheless, much remains to be unraveled about the physical, chemical, and biological basis for many diseases, particular those affecting the brain. Moreover, it has become clear that genetic factors are incomplete for describing many normal and aberrant processes, indicating an important contribution from environmental factors, whether they derive from the particular palette of components specific to different cell types, the unique tissue microenvironment, or agents encountered by the organism writ large.

An excellent example of this challenge concerns the protein α-synuclein. An abundant protein expressed predominantly in neurons, α-synuclein gained notoriety in 1997 when genetic and pathological studies implicated it in Parkinson?s disease, which affects at least 500,000 people in the United States alone. In Parkinson?s disease, α-synuclein misfolds and aggregates into toxic protein assemblies that can cause neuronal death. How α-synuclein contributes to neurodegeneration remains unclear and controversial, owing in part to an incomplete understanding of its biophysical properties and cellular interactions. Though mutations to α-synuclein can cause Parkinson?s disease, most Parkinson?s cases have little or no genetic basis. In addition, though expressed widely throughout the brain, α-synuclein pathology impacts only a small subset of neurons, indicating a strong contribution from cellular context. How this protein interacts with its cellular environment is therefore of significant interest for understanding the etiology of Parkinson?s disease.

In this course, we will examine how environmental factors affect α-synuclein misfolding and toxicity with the goal of clarifying how α-synuclein contributes to neurodegeneration.

Suggested Reading

Bendor, J. T.; Logan, T. P.; Edwards, R. H. The function of α-synuclein [3]. Neuron 2013, 79,
Course Description

The centerpiece of this course is an interdisciplinary research project that will be completed in teams. Students will perform the experiment, collect and analyze the data, and draw conclusions. Though extensive support will be provided by faculty and instructional staff, students will be encouraged to explore and execute their own ideas. The results thereby obtained will be integrated into a manuscript for peer-reviewed publication. Course content will be delivered through a combination of lectures from guest faculty, technique-focused talks from instructional staff, and literature reviews by students. Students will also present oral progress reports and give a final oral presentation of their findings for a wide audience.

Course Goals

The goal of the course is to provide an immersive, hands-on experience in the context of genuine research questions. As articulated by Vale and colleagues (http://www.sciencemag.org/content/338/6114/1542.long [8]), there are tremendous advantages when graduate students work "pursuing a research question with unknown answers and uncertain outcomes, students and faculty combine their wits and skills to design experiments, evaluate progress, and troubleshoot along the way". These advantages are likely to be common across all learning levels (http://blogs.kqed.org/mindshift/2014/09/can-project-based-learning-close-gaps-in-science-education/ [9]). In our course, teams may use whatever literature, software, and resources that are available publicly, and are encouraged to write their own scripts and software where necessary.

This course will introduce students to approaches and methodologies for interrogating biological systems in high throughput, which will require the integration of experiment and computation. In addition to fundamental techniques in modern molecular biology and bioinformatics, students will learn to interpret and leverage large datasets, draw original conclusions, and present findings in written and oral formats.

The "official" language of the class is python (https://www.python.org [10]) - beginners should try Learn Python The Hard Way (http://learnpythonthehardway.org/book/ [11]), people with a background in other languages should try Google's python course (https://developers.google.com/edu/python/ [12]). The QB3 Berkeley intensive python course (http://intro-prog-bioinfo-2014.wikispaces.com/ [13]) provides many biological examples. Students should be comfortable with basic syntax and scripting prior to the start of instruction.
Student Learning Objectives

- Laboratory safety
- Scientific documentation
- Experimental design
- Yeast manipulation
- Molecular biology techniques
- Library preparation
- Deep sequencing
- Bioinformatics
- Computer programming
- High-content microscopy
- Image processing
- Biophysical computation

Class Policies

**Ethics:** This course is more than a training experience; it is an active research project whose results will be published to the broader scientific community. The community must be able to understand our work, replicate it, and have confidence in its findings. We must therefore ensure the integrity of the information we disseminate. To do so, it is essential that students perform and document their experiments and analyses as faithfully as possible. Mistakes and oversights are normal and to be expected, but they must not be ignored, concealed, or disguised. In addition, to merit authorship, students must contribute to three aspects of the project: intellectual conception or interpretation of the methods or data, technical execution of the experiments and/or analyses, and documentation or dissemination of the results. We fully expect that by actively participating in the course and working toward the course objectives, all students will merit authorship.

**Respect:** This course is built around an open research project performed in teams. Successful completion of the course objectives will require that students work together effectively, so please respect the time and effort of your classmates and instructors. Moreover, as part of the research process, we will consider and debate a variety of ideas and approaches; however, we must not allow our position on a particular idea or argument to compromise our respect for its author. We therefore expect course participants to give all instructors and students, regardless of academic or personal background, their complete professional respect; anything less will not be tolerated.

**Absences:** The instructor must be notified by the second week of classes for any planned absences, or in advance of class due to illness. Active participation in the laboratory is essential and students are required to attend normal class hours. Occasional attendance outside of regular class hours will also be necessary, as indicated by the syllabus. Attendance during the final presentation is absolutely mandatory, except in cases of doctor-excused
medical illness. Any class material or lecture that is missed will be the responsibility of the student. Unexcused absences may affect the final course grade. Written evaluations of each team and its members will be provided to the Graduate Tracking System for inclusion into the graduate record, and provided to oral committee members and thesis committee members.

**Accommodations for students with disabilities:** The Graduate Division embraces all students, including students with documented disabilities. UCSF is committed to providing all students equal access to all of its programs, services, and activities. Student Disability Services (SDS) is the campus office that works with students who have disabilities to determine and coordinate reasonable accommodations. Students who have, or think they may have, a disability are invited to contact SDS (StudentDisability@ucsf.edu [15]); or 415-476-6595 for a confidential discussion and to review the process for requesting accommodations in classroom and clinical settings. More information is available online at [http://sds.ucsf.edu](http://sds.ucsf.edu)[16]. Accommodations are never retroactive; therefore students are encouraged to register with Student Disability Services ([http://sds.ucsf.edu](http://sds.ucsf.edu))[17] as soon as they begin their programs. UCSF encourages students to engage in support seeking behavior via all of the resources available through Student Life, for consistent support and access to their programs.

**Schedule**

**Week 1 ? Orientation, perturbation selection, growth optimization**

*Monday, September 18*

Lecture: Introduction and Course Goals, by Martin Kampmann

[ PUBS_2017-Intro.pdf](http://example.com)[18]

Lecture: Introduction to a-Synuclein, by Robert Newberry

[170918 PUBS Intro Slides.pdf](http://example.com)[19]

Labwork: Choose team names, select chemical perturbants, plan growth experiments

Group Presentation: Compound Choice

Computation: Set up cluster access

*Tuesday, September 19*

Tech Talk: Organizing Experiments, by Taylor Arhar
Tech Talk: Aseptic Technique, by Dan Schwarz

Aseptic Technique.pptx

Protocol Talk (George): Growth Experiments

Labwork: Preculture for growth experiment; prepare media

8pm: Induce expression

Wednesday, September 20

8:30am: Collect growth data

Lecture: Yeast as a Model Organism, by Hiten Madhani

Labwork: Analyze growth data - 1 minute presentation from each group on your results

Tech Talk: GitHub and Version Control, by Alison Maxwell

GitHub_presentation.pdf

github-git-cheat-sheet.pdf

Git_setup_terminaloutput.pdf

Tech Talk: Yeast Plasmids and Expression, by Doug Wassarman

PUBS_Plasmids.pptx

Computation: Barcode association for ubiquitin

Files for Computation:
- Allele_dic.pkl
- translate.pkl
- aminotonumber.pkl

Folder

Pickle.pdf
Week 2 ? Library selection

Monday, September 25

Lecture: Proteostasis, by Jason Gestwicki

Team presentations: Barcode Analysis

Lecture: Chemical Genetics, by Martin Kampmann


Protocol Talk (Erik): Selection experiments [34]

Labwork: Prepare media for selection experiments

8pm: Induce expression, replicate 1

Tuesday, September 26

8:30am: Collect miniprep samples, replicate 1 timepoint 1

Lecture: Biology of ?-Synuclein, by Robert Edwards


Labwork: Growth selection for replicate 1; Preculture for replicate 2

Computation: a-Synuclein barcode analysis

Log into fastq.ucsf.edu. The path to the folder containing your files is:

data1/temp/170922_M00582_0216_000000000-B673F/fastq1/

There are three gzipped fastq files; their names contain R1, R2, R3 (read 1, read 2 = barcodes, read 3)
Here is the nucleotide sequence of wild-type a-synuclein in the plasmid:

ATGGATGTATTCTGGAGGACCTTTCAAGGCCAAGGAGGGAGTTGCTGCTGCTGAGAAAACCAAACAGGGTGTGGCAGAAGCAGCAGGAAAGACAAAAGAGGGTGTTCTCTATGTAGG ... TGGGCAAGAATGAAGAAGGAGCCCCACAGGAAGGAATTCTGGAAGATATGCCTGTGGATCCTGACAATGAGGCTTATGAAATGCCTTCTGAGGAAGGGTATCAAGACTACGAACCTGAAGCC

8pm: Collect miniprep sample, replicate 1 timepoint 2
8pm: Induce expression, replicate 2

Wednesday, September 27

8:30am: Collect miniprep sample, replicate 2 timepoint 1
Lecture: Gene synthesis, by Charles Joseph
Labwork: Growth selection for replicate 2
Computation: Alpha-synuclein barcode analysis
8pm: Collect miniprep sample, replicate 2 timepoint 2

Week 3 ? Microscopy, molecular biology

Monday, October 2
Lecture: Next-Generation Sequencing, by Eric Chow
Protocol Talk (Matt): Yeast miniprep [38]
Labwork: DNA miniprep
Team presentations: Alpha-synuclein barcodes

Tuesday, October 3
8:30am: Induce expression

Lecture: Microscopes and Image Acquisition, by DeLaine Larson

Protocol Talk (Snow): Microscopy [40]

Protocol Talk (Maureen): PCR Overview [41], PCR Day 1 [42], PCR Day 2 [43]

Labwork: PCR Day 1 [42]; imaging

Wednesday, October 4

Lecture: Image Analysis, by Sy Redding

Images from Sy:
https://drive.google.com/open?id=0B7hDRBE4CxxMQ09jbnZlY0N4WVE [44]
https://drive.google.com/open?id=0B7hDRBE4CxxMcXB3Q0RwME96Sm [45]

Script from Sy:
https://docs.google.com/document/d/11k7MySuDExCEAWMXH-wkQ5dsZVz5tid8LhkotslC-0/edit?usp=sharing [46]

Labwork: PCR Day 2 [43], sample pooling and submission

Computation: Begin image analysis

Week 4: Biophysical computation, sequence analysis

Monday, October 9

Lecture: Structural Biology of Amyloids, by Bill DeGrado


Computation:
- Download new paired end data (alpha-synuclein barcode mapping) from fastq.ucsf.edu here:
/data1/temp/171007_M02564_0094_000000000-BFP9Y/FASTQ/
- Download screen results from fastq.ucsf.edu here:
/data2/171005_K00153_0442_BHLNKCBBXX_PUBS/PUBS_2017/
- The 50bp HiSeq reads contain the following constant sequence after the 26bp barcode: GAGCTCTCTAGAGGGCCGCATCATG

- Generate barcode dictionary

Around 4 pm today, each team will present their preliminary results and strategy for image analysis: features to be quantified, and who does what

**Tuesday, October 10**

Lecture: Biophysical Computation, by Tanja Kortemme


Computation: Microscopy analysis, Fitness calculations

**Wednesday, October 11**


Computation: Continue analysis.

At 3:30 pm: groups present their results so far, and plans for next steps

**Week 5 ? Data Analysis**

**Monday, October 16**


Work in subgroups

**Tuesday, October 17**

Journal Club (Kyle): Toth-Petroczy, et al. Structured States of Disordered Proteins from Genomic Sequences
Tech Talk (Taia): Introduction to PyMOL

Work in subgroups

Wednesday, October 18

Journal Club (Lakshmi): Hughes et al. Low-complexity domains adhere by reversible amyloid-like interactions between kinked \(\beta\)-sheets. on BioRxiv

Subgroup presentations (beginning of class)

Work in original teams

Week 6 - Data analysis, presentation preparation

Monday, October 23

Robert: Presentation of background slides that will be shown before your presentations on Thursday

Work in teams on presentations

Tuesday, October 24

Practice presentations around 3:30 pm (faculty will visit each team for this)

Wednesday, October 25

Work in teams on finalizing presentations

Thursday, October 26

3PM: Final Presentations