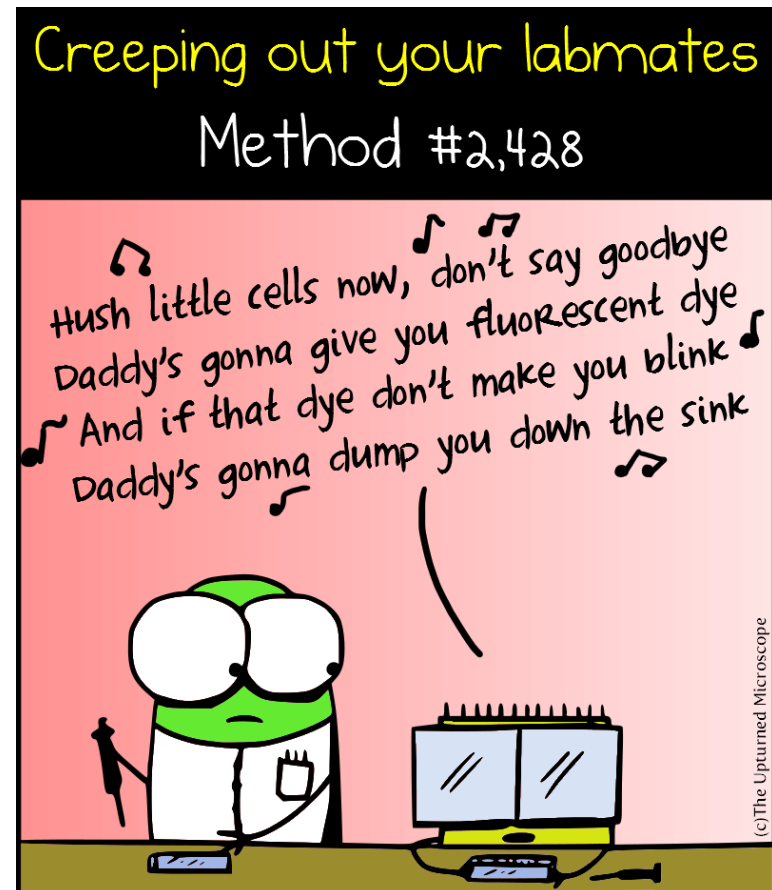


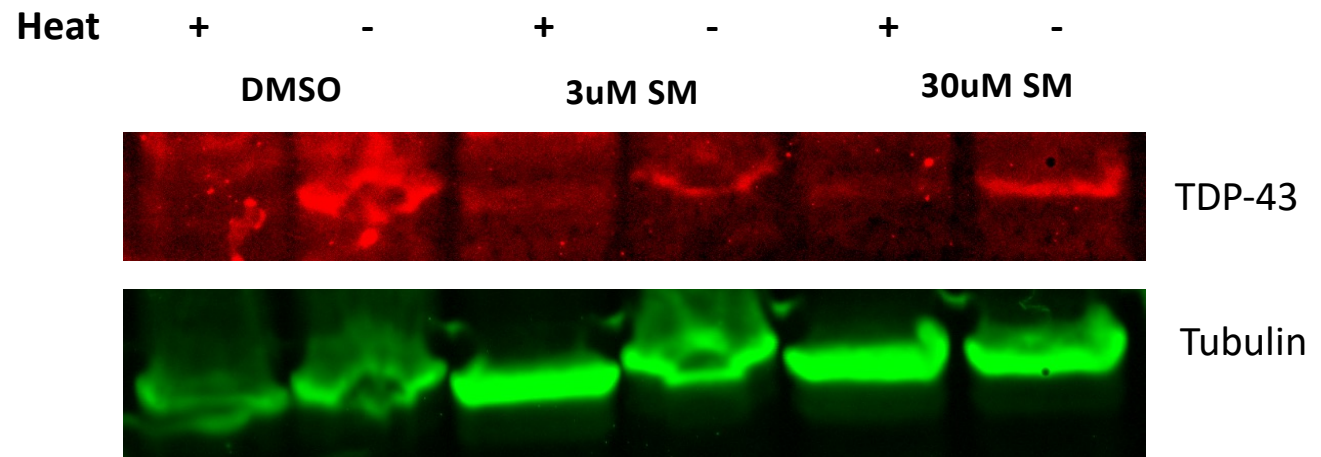
M3D1: Review IPC literature and examine structural characteristics

- Prelab discussion
- Examine literature, sequence, and protein structure of inverse pericam (IPC)



CETSA review

- What do the bands (or lack thereof) mean?
- How could you compare across groups? (multiple ways)
- Did the CETSA “work” as expected?
 - Did the small molecules?

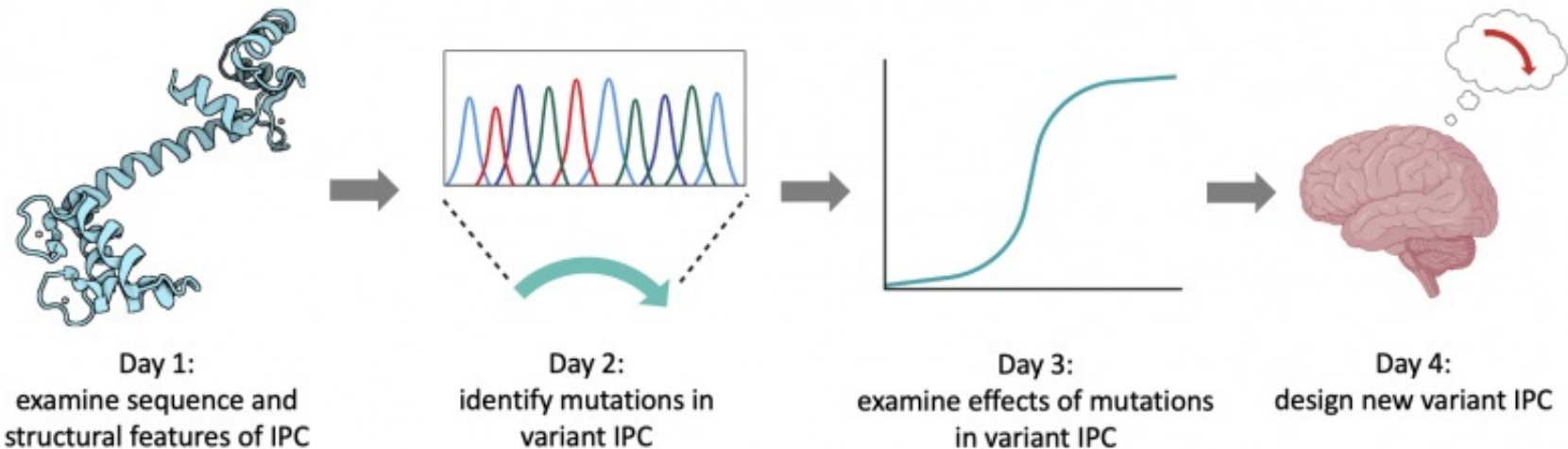


Important Mod 3 Due Dates

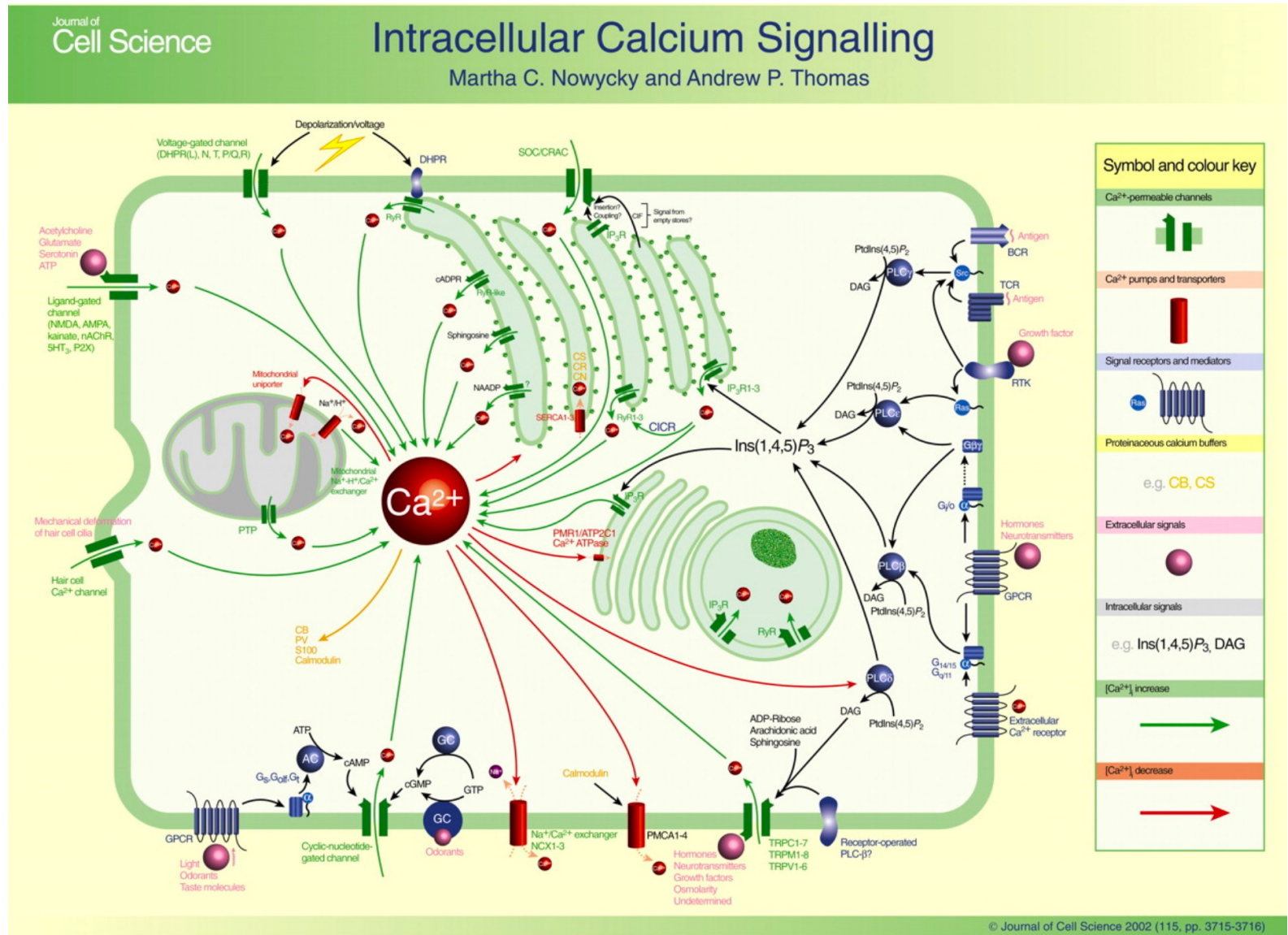
- **Research proposal presentation** (20%)
 - completed in teams and presented via Zoom
 - due 5/19
- **Mini-report** (5%)
 - completed in teams and submitted via Stellar
 - due 5/14 at 10p
- **Quizz** (collectively 10%)
 - M3D4
- **Notebook** (part of 10% Homework and Notebook)
 - due 4/12 at 10p
- **Blog** (part of 5% Participation)
 - due 5/20 at 10p via Slack (unless you have already completed 3 posts)

Mod3 Experimental Overview

Research goal: Perform site-directed mutagenesis to alter the properties of a protein-based fluorescent sensor



What do we want to study?



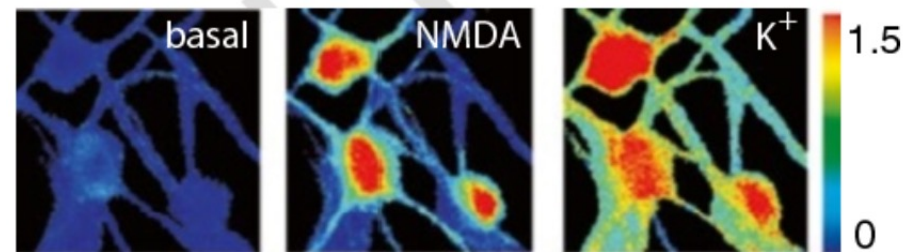
Why calcium signaling?

- Calcium signaling is an essential second messenger and signal transducer
- Large dynamic range, but can be toxic at high concentrations
- Often used to indicate “activity”

Calcium reveals connections between neurons

New way to image brain-cell activity could shed light on autism and other psychiatric disorders.

Anne Trafton, MIT News Office



How can we create a calcium sensor?

Utilize a protein that binds calcium as a sensor

- Calmodulin (CaM)
 - Calcium modulated protein
 - 4 calcium binding sites
 - Changes conformation upon calcium binding
- Effector protein activated by calcium

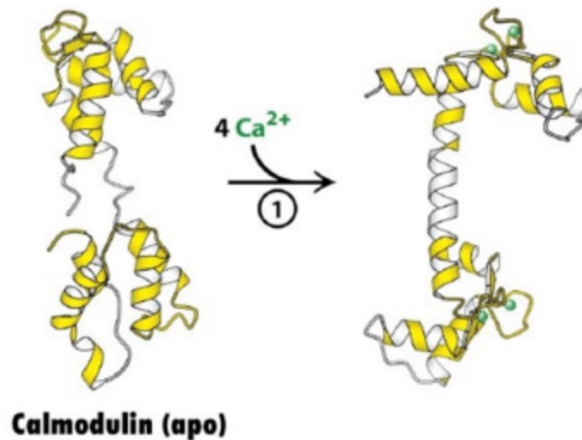
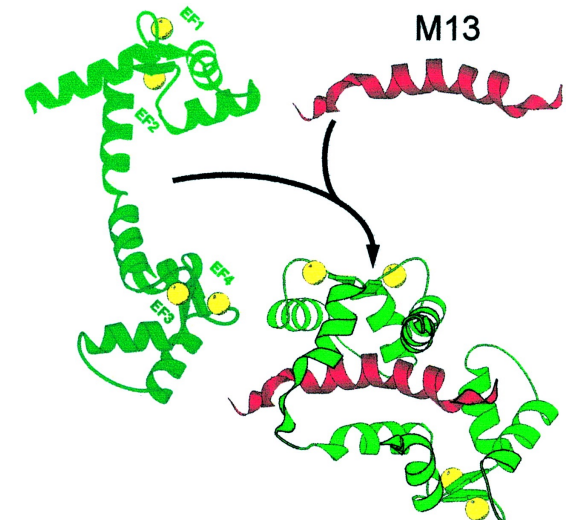


Figure 14-16b
Biochemistry, Sixth Edition
© 2007 W. H. Freeman and Company

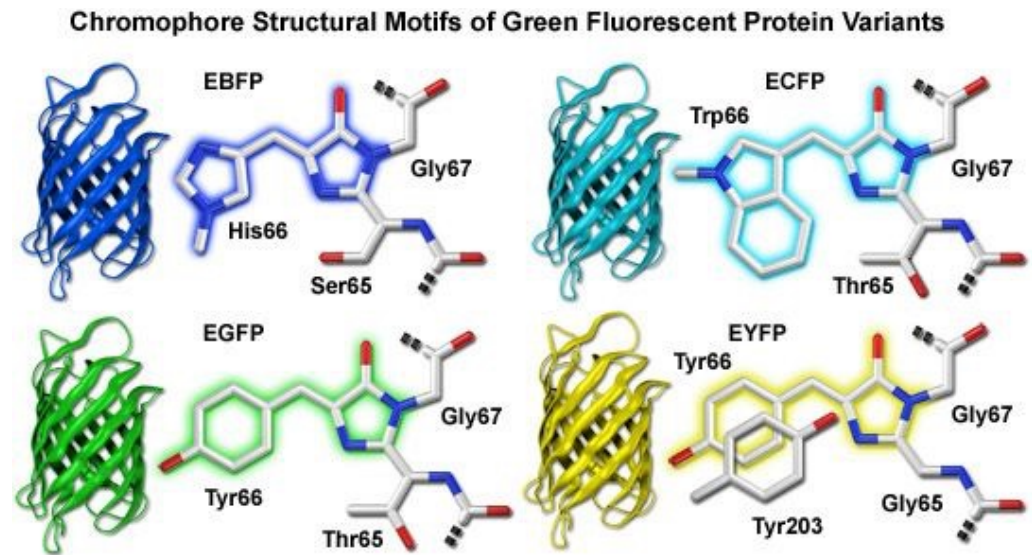


Carafoli. PNAS. 2002

- M13
 - Synthetic peptide derived from myosin light chain kinase
 - Target peptide for CaM
 - Promotes further conformation change in CaM bound to calcium

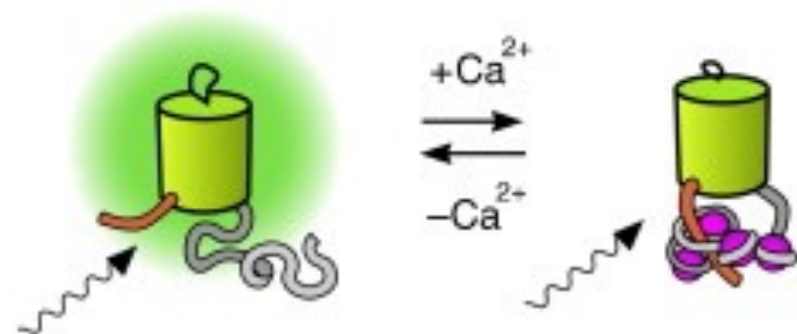
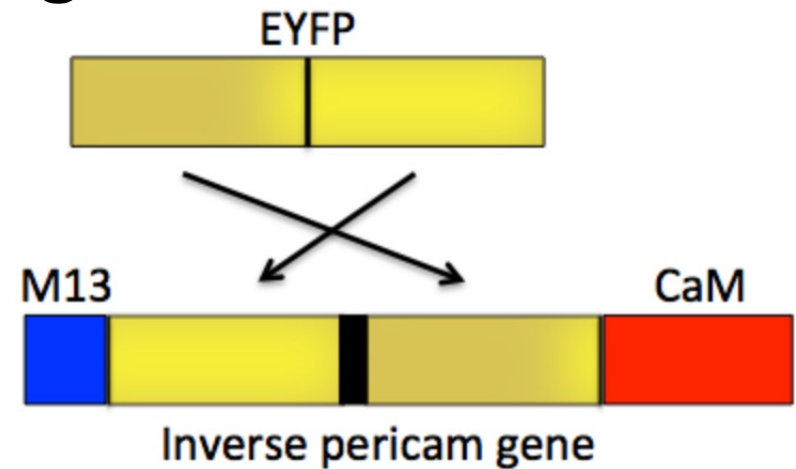
How can we visualize calcium binding?

- Fluorescence!
 - Enhanced yellow fluorescent protein (EYFP)
 - Mutant of GFP
- Why would fluorescence be a good way to visualize our sensor system?



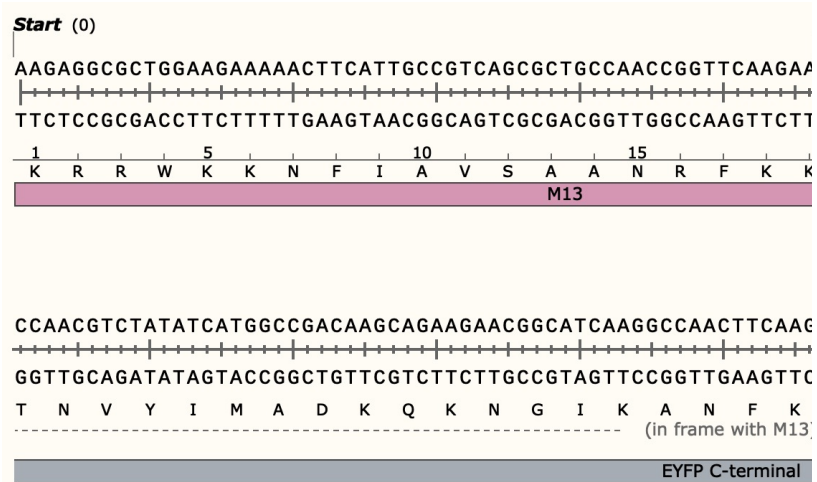
What sensor are we modifying?

- Inverse pericam (IPC)
- Fluorescence dims upon Ca^{2+} binding
- We can use a titration curve and our fluorescent readout to quantify calcium binding
- Design and test point mutations to alter CaM binding to Ca^{2+}

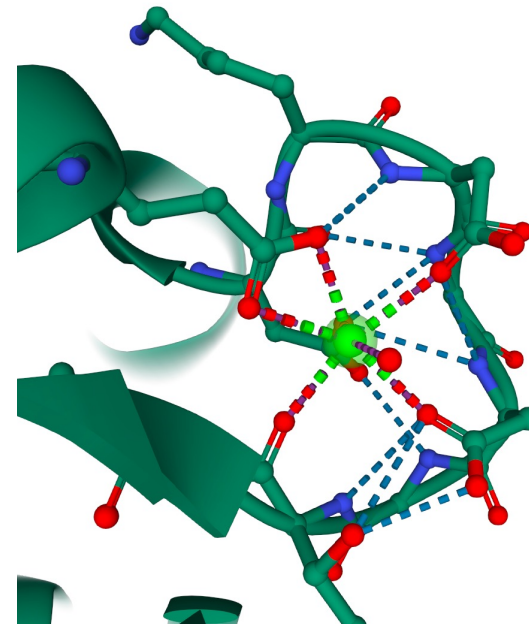


What are you examining today?

- Sequence

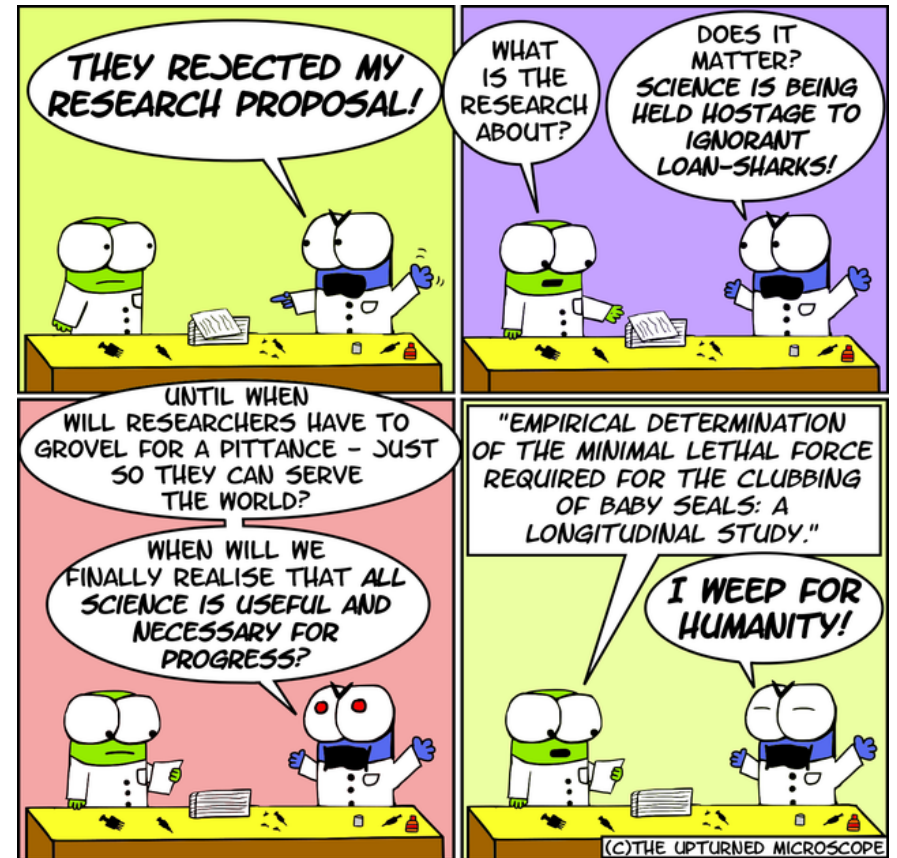


- Structure



Setting up a research proposal

- Identify work you find interesting and important
 - Can be any aspect of biological engineering
 - Must be tangentially related to '109
- Think about how you could expand on that work or apply it to new topic



For Today

- Identify sequence features and structural elements of IPC

For M3D2...

- Describe 5 recent articles with interesting findings that could be developed for your Research Proposal Presentation
 - Include citation information for each article
 - Write 3-5 sentences that summarize the key finding
 - **Individual homework assignment**