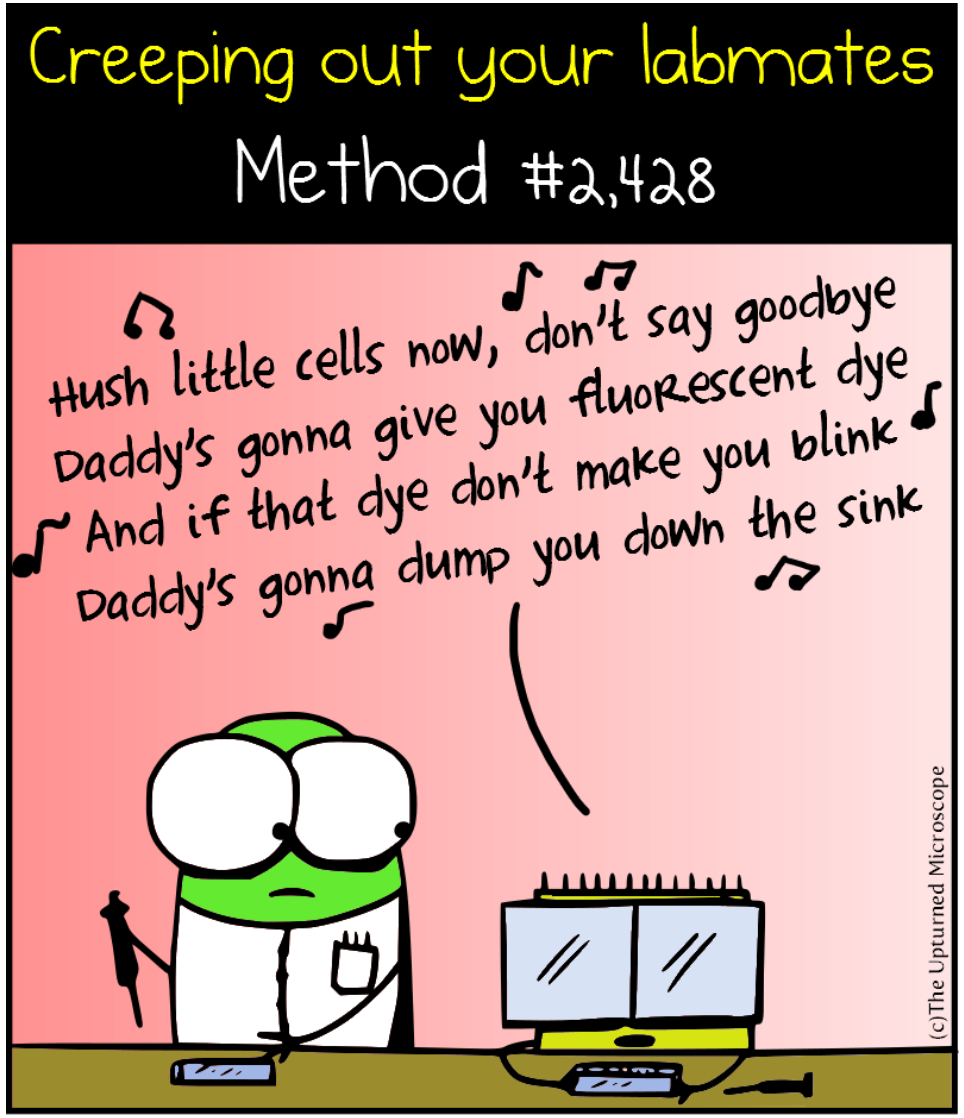
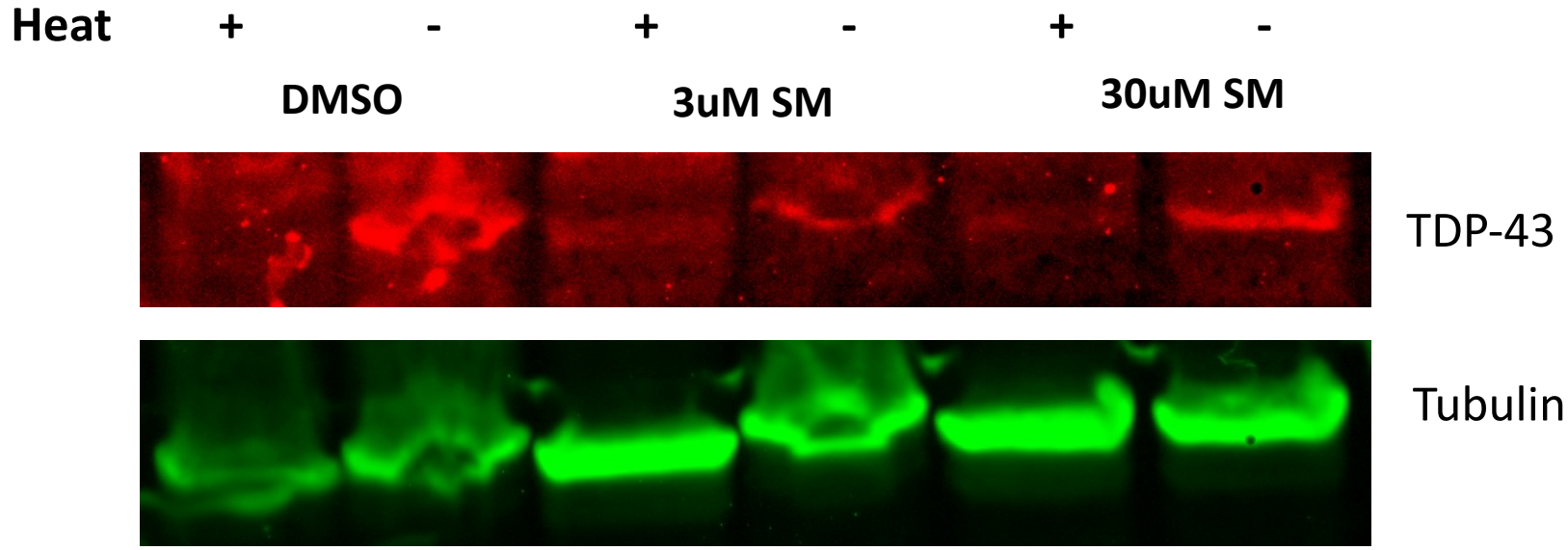
M3D1: Review IPC literature and examine structural characteristics

- 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?

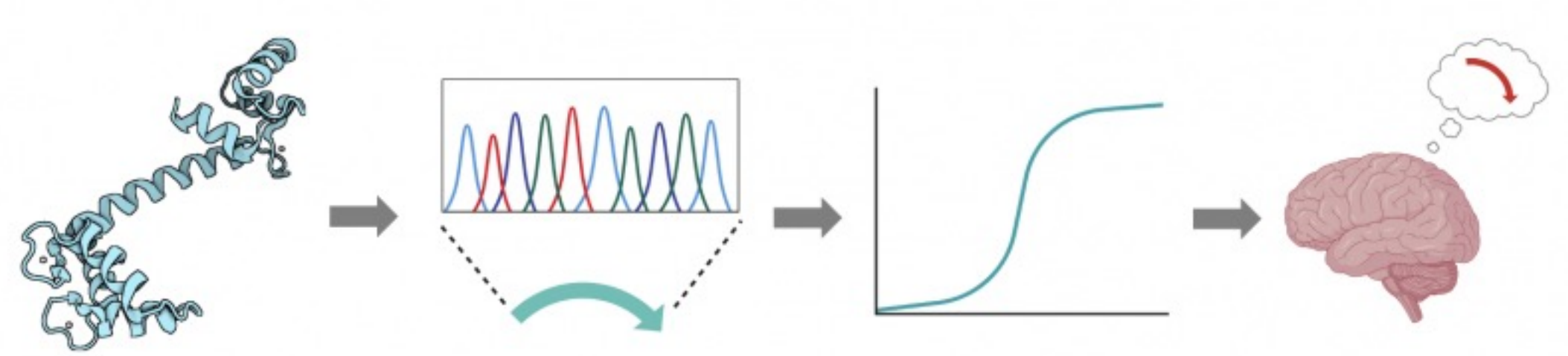


Important Mod 3 Due Dates

- Research proposal presentation (20%)
  - completed in teams and presented via Zoom
  - due 5/18
- Mini-report (5%)
  - completed in teams and submitted via Stellar
  - due 5/13 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



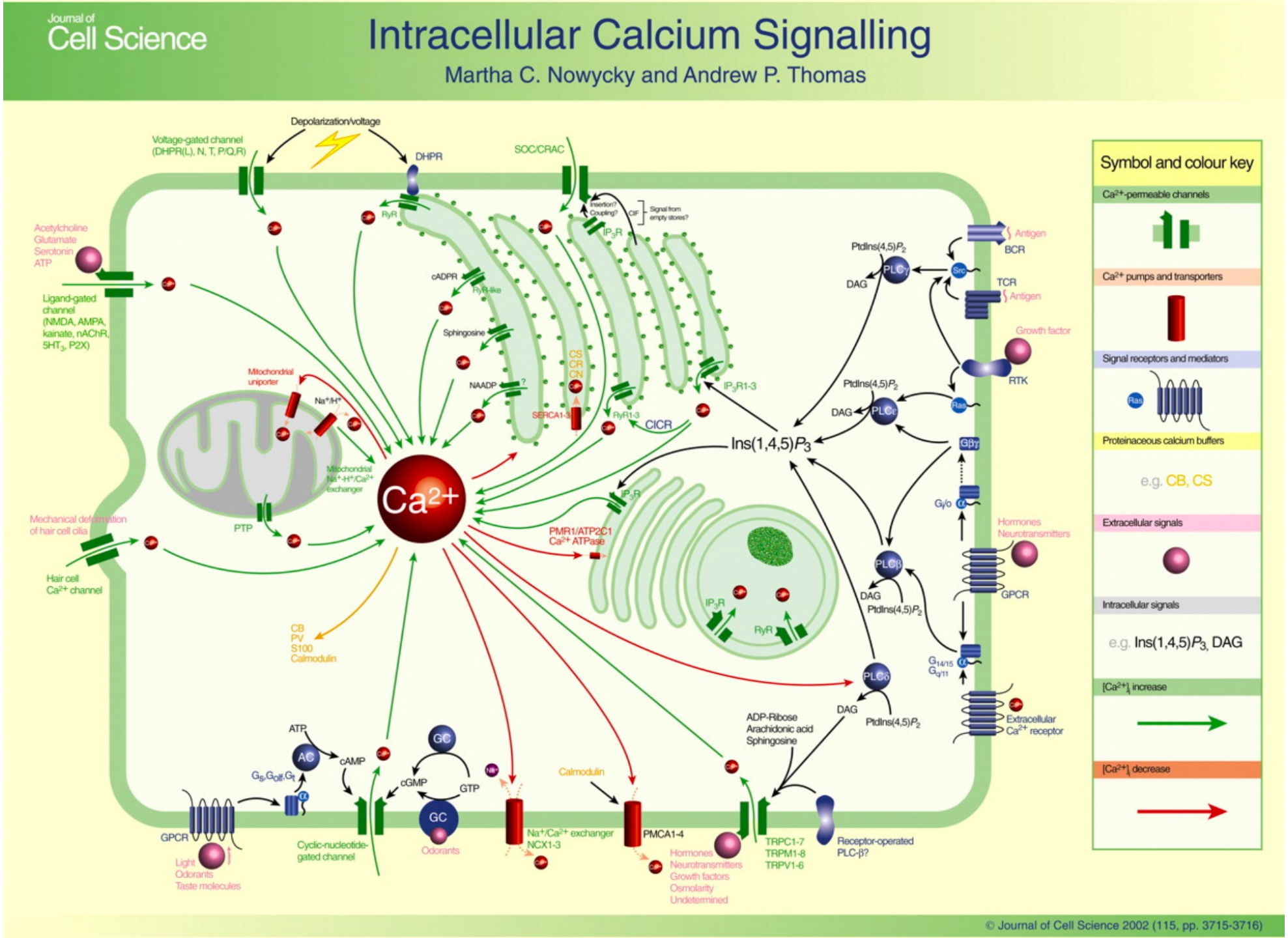
Day 1:
examine sequence and structural features of IPC

Day 2:
identify mutations in variant IPC

Day 3:
examine effects of mutations in variant IPC

Day 4:
design new variant IPC

What do we want to study?



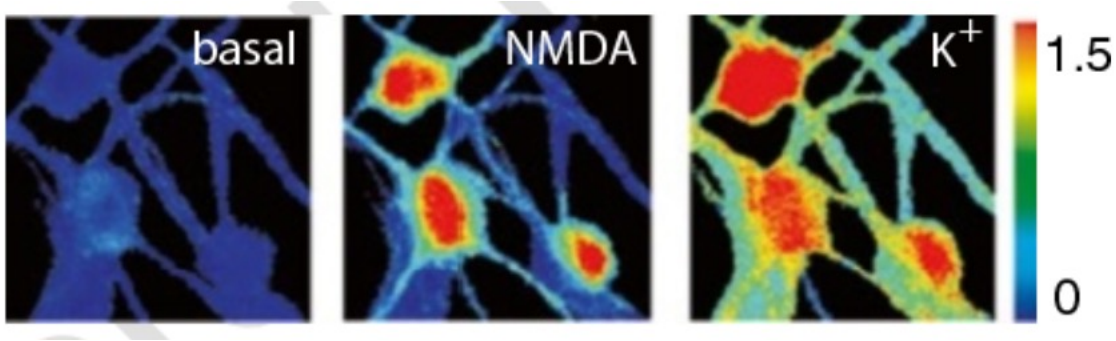
Why calcium signaling?

- 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.

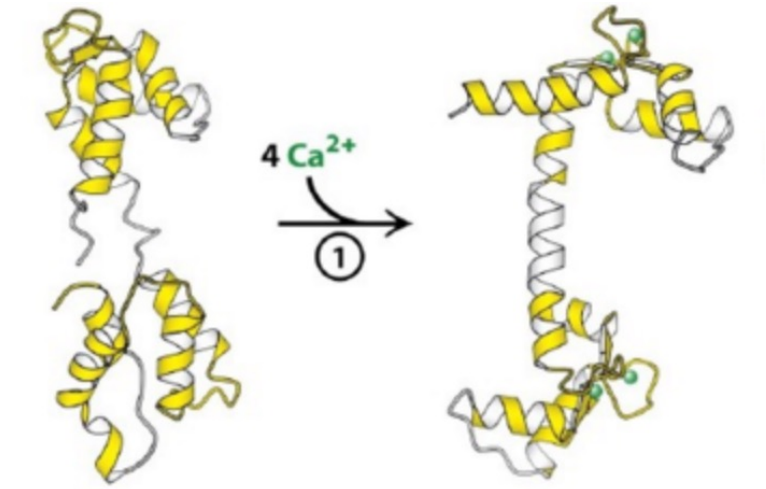
Anne Trafton, MIT News Office



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



Calmodulin (apo)

Figure 14-16b
Biochemistry, Sixth Edition
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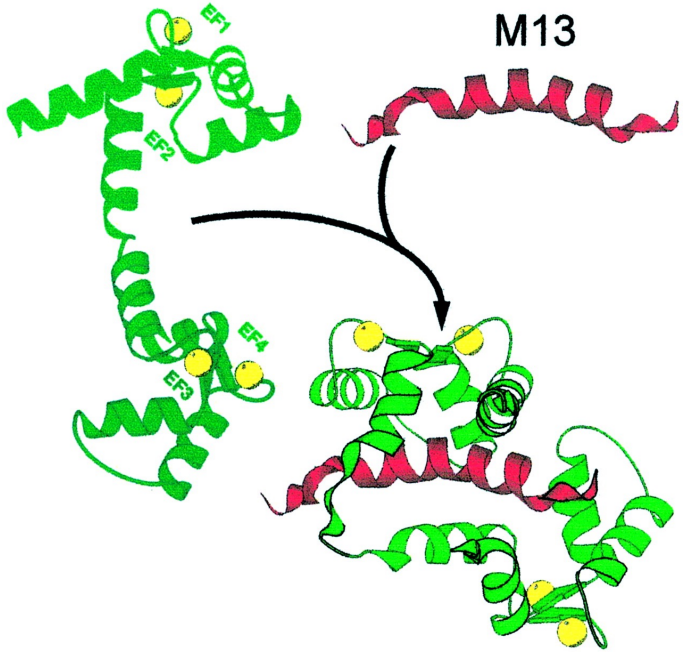
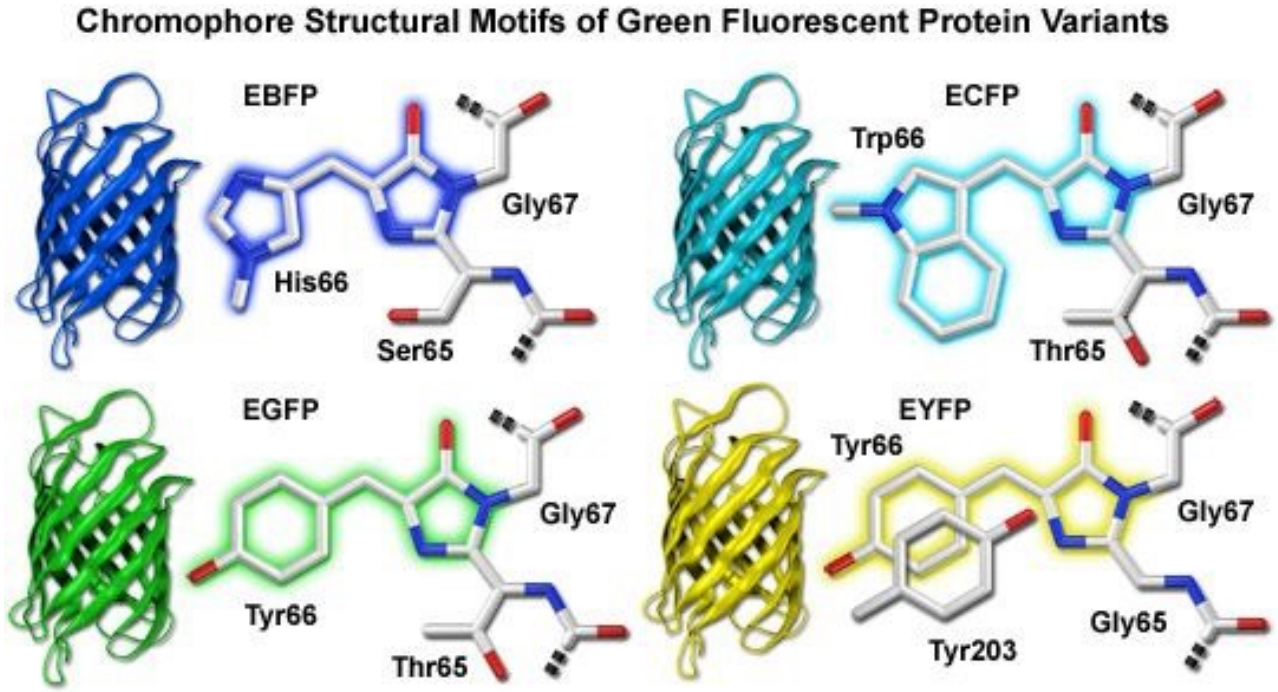


Carafoli. PNAS. 2002

- 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?

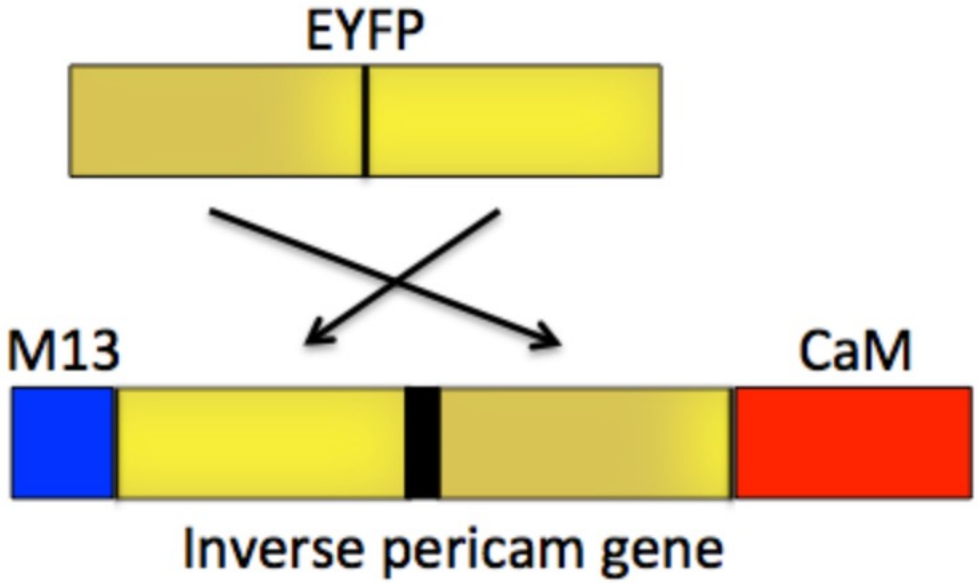
- Fluroescence!
  - Enhanced yellow fluorescent protein (EYFP)
  - Mutant of GFP
- Why would fluorescence be a good way to visualize our sensor system?

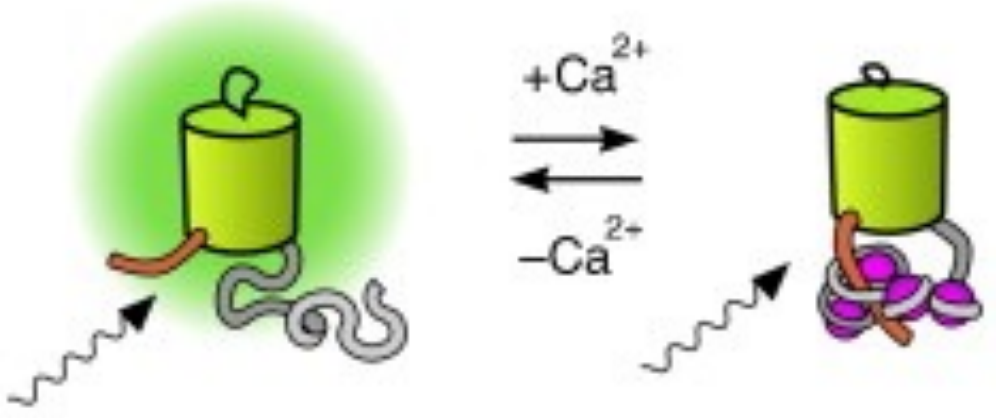


Olympus

What sensor are we modifying?

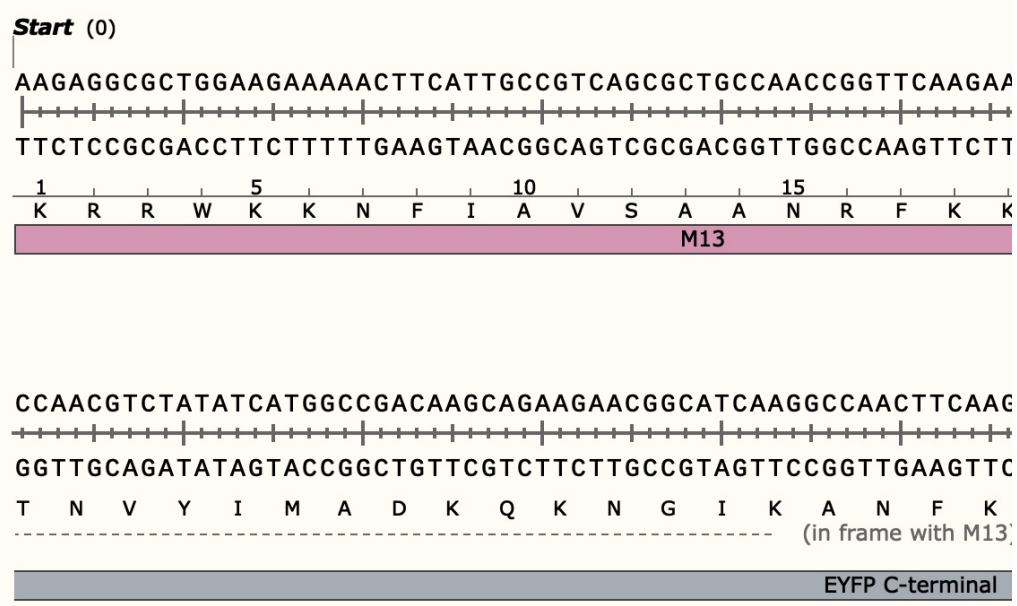
- Inverse pericam (IPC)
- Fluorescence dims upon Ca^{2+} binding
- We can use a titration curve and our fluorescent readout to quantify calcium binding
- Design and test point mutations to alter CaM binding to Ca^{2+}



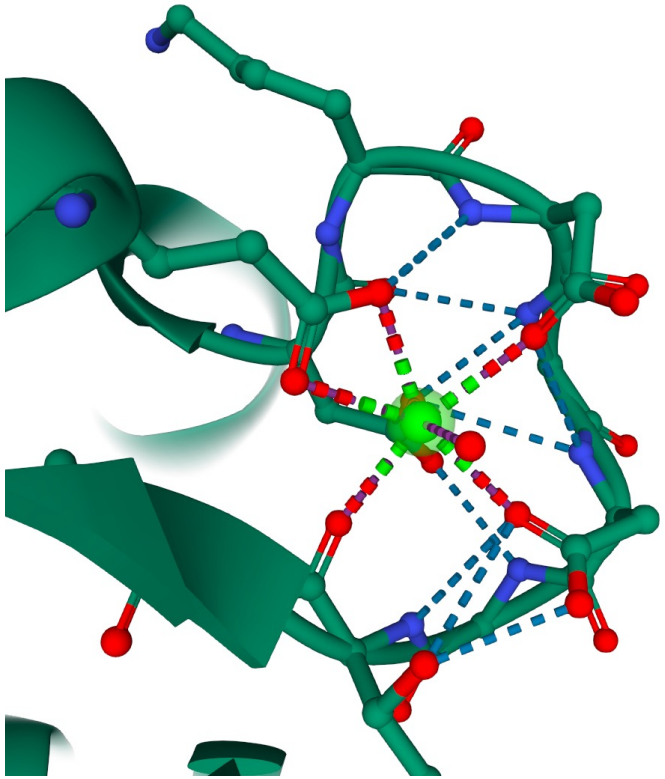


What are you examining today?

- Sequence

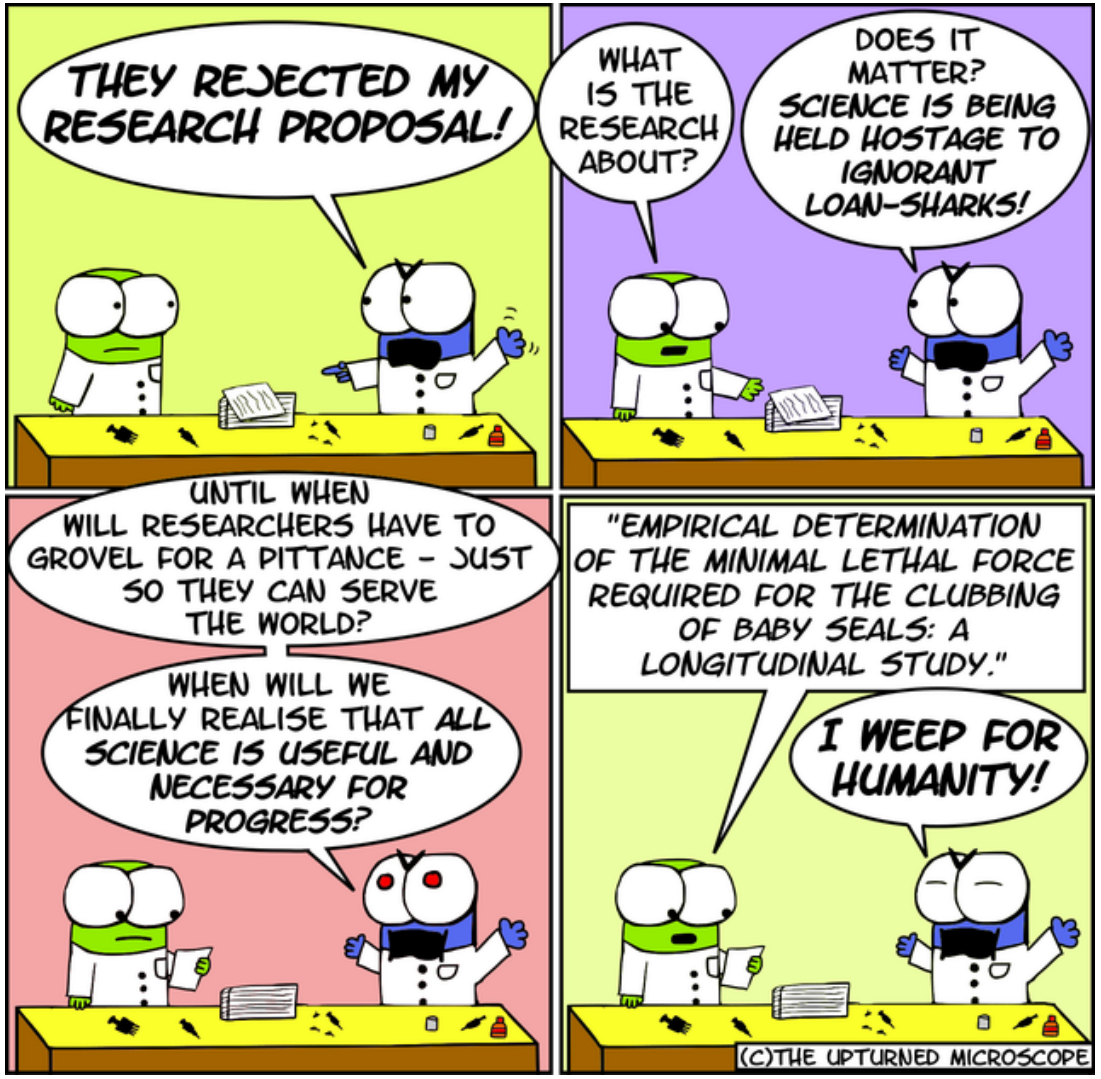


- Structure



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
  - Done individually