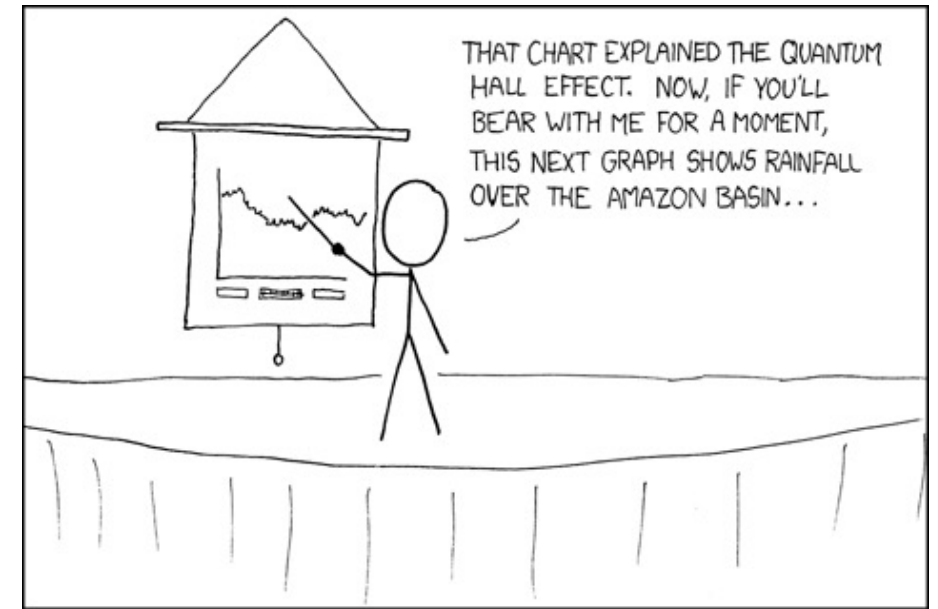


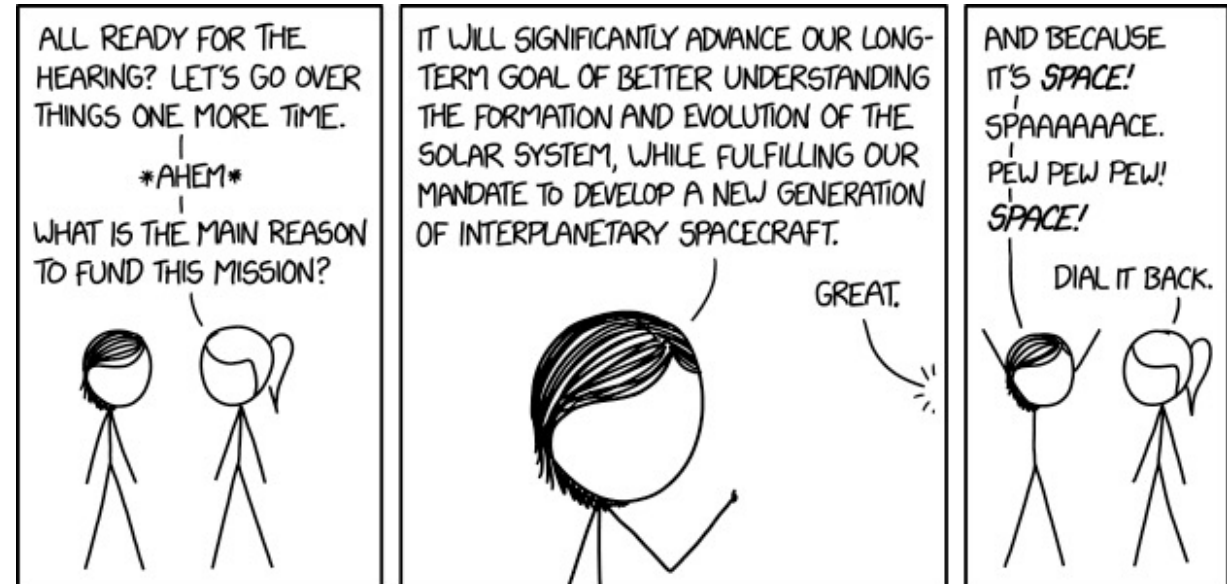
# M3D4: Design new IPC variant

- Prelab discussion
- Determine improved IPC variant and design mutation primers
- Quiz beginning at 3:15pm

\*\* Will use the societal/ethical implications in proposal



IF YOU KEEP SAYING "BEAR WITH ME FOR A MOMENT", PEOPLE TAKE A WHILE TO FIGURE OUT THAT YOU'RE JUST SHOWING THEM RANDOM SLIDES. xkcd

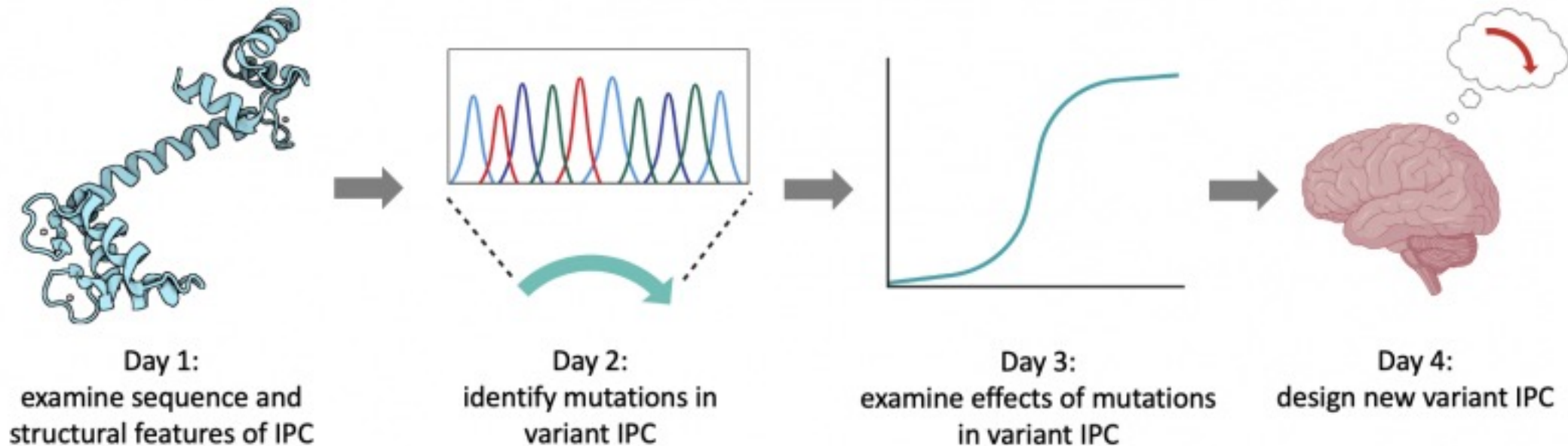


# Important Mod 3 Due Dates

- **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
- Quiz (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)

# Mod 3 Overview

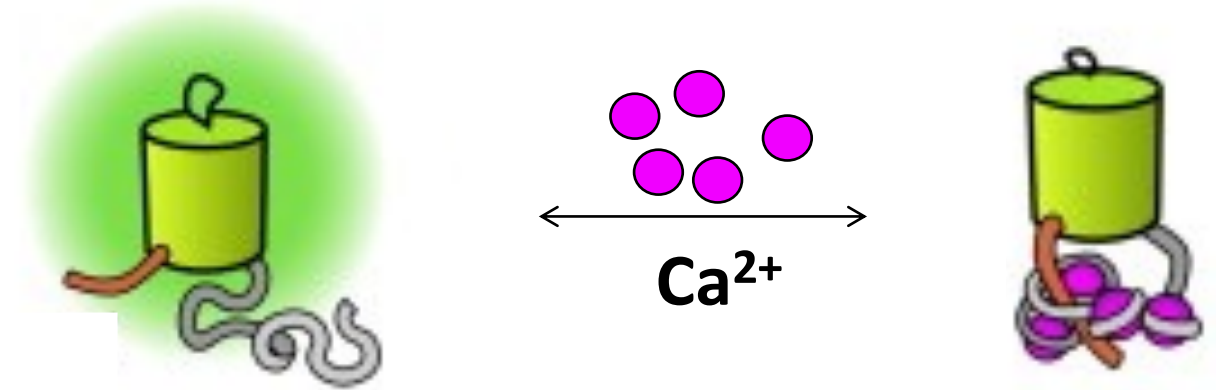
**Research goal: Perform site-directed mutagenesis to alter the properties of a protein-based fluorescent sensor**



# Create a new IPC variant

Bringing everything together!

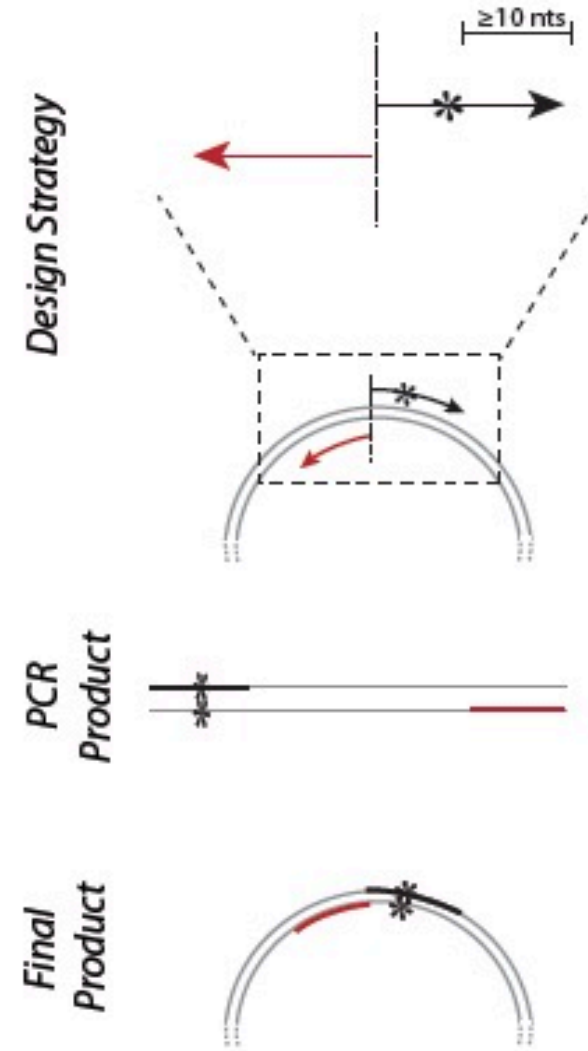
- Based on:
  - Structure
  - Sequence
  - $\text{Ca}^{2+}$  binding curves of previous mutations



What amino acid on in the calmodulin sequence of IPC would you target to alter binding to calcium?

# Use site-directed mutagenesis (SDM) to engineer plasmid DNA

- NEB Q5 SDM kit
- Create specific, targeted changes in double-stranded plasmid DNA
- Forward primer:
- PCR product:
- Final product:

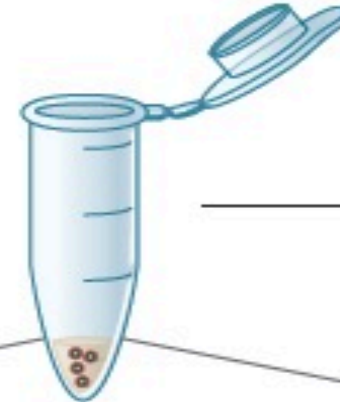
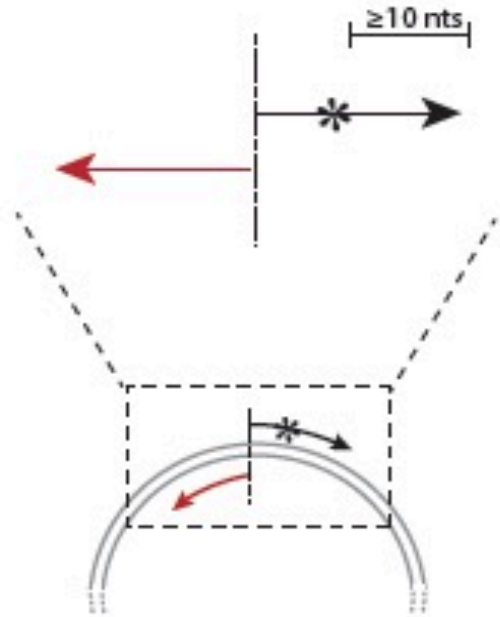


# SDM Part 2: Recover circular plasmid product using Kinase-Ligase-Dpn1 (KLD) enzyme mix

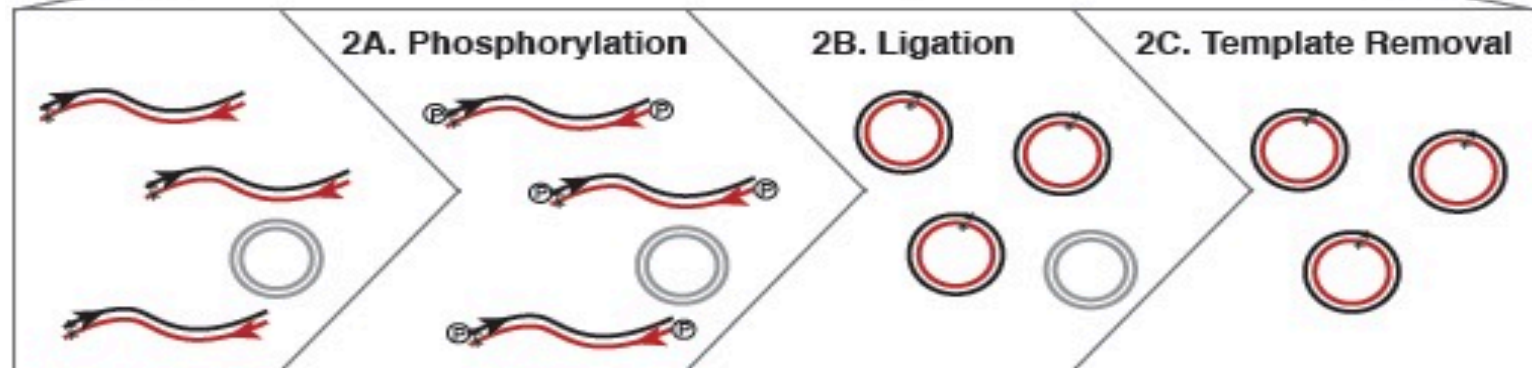
1. Exponential Amplification (PCR)
  - Q5 Hot Start 2x Master Mix

2. Treatment & Enrichment: Kinase, Ligase and DpnI
  - 10x KLD enzyme mix

3. High-efficiency transformation
  - NEB 5-alpha competent cells



5 min. at room temp.



# For Today:

- Design optimized primer to alter calmodulin binding to calcium
- Finish lab notebooks (due 5/12 by 10pm)
- Work on mini-report
- Quiz at 3:15pm



**M3D3**

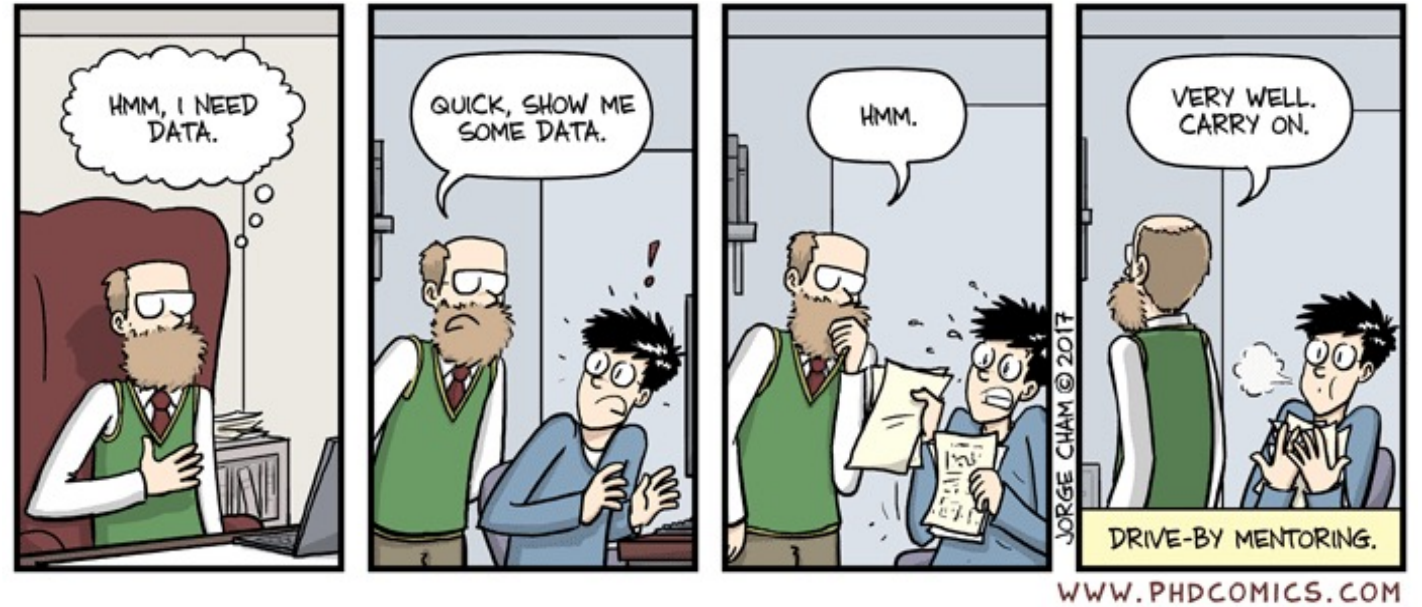
Matlab trouble?  
Talk to me...

# Mini-Report: as always, read the wiki...

- 2-3 pages max!
  - Completed in teams
- Audience has high familiarity with your project
- What figures will you include?



# For Thursday



- Thursday lab is entirely devoted to completing the mini-report
  - Should be able to complete it during lab, due at 10pm

# This is it!

- It's been quite a semester...
  - Thank you for patience and engagement during a challenging time
- Feedback session on May 20 during lecture

