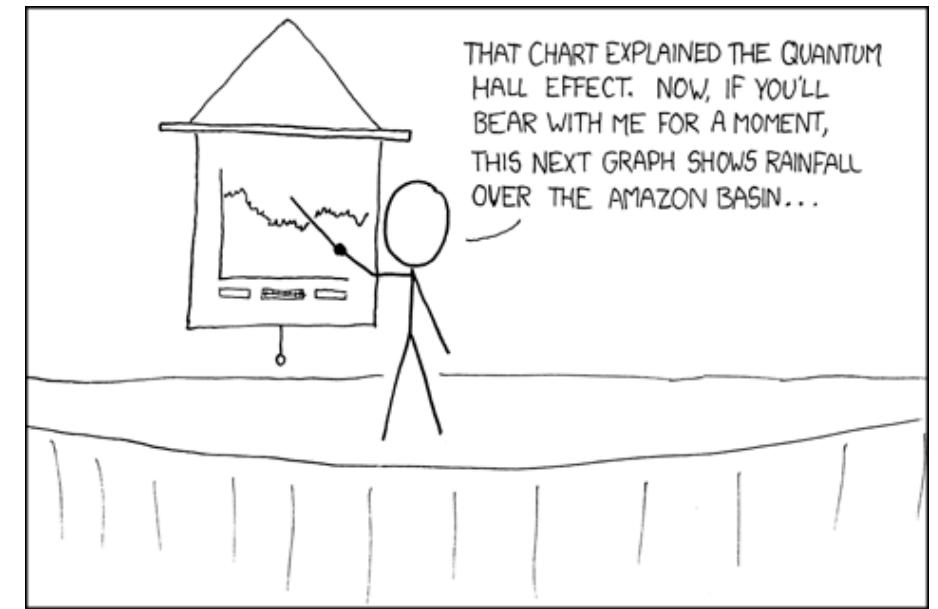


# M3D4: Design new IPC variant

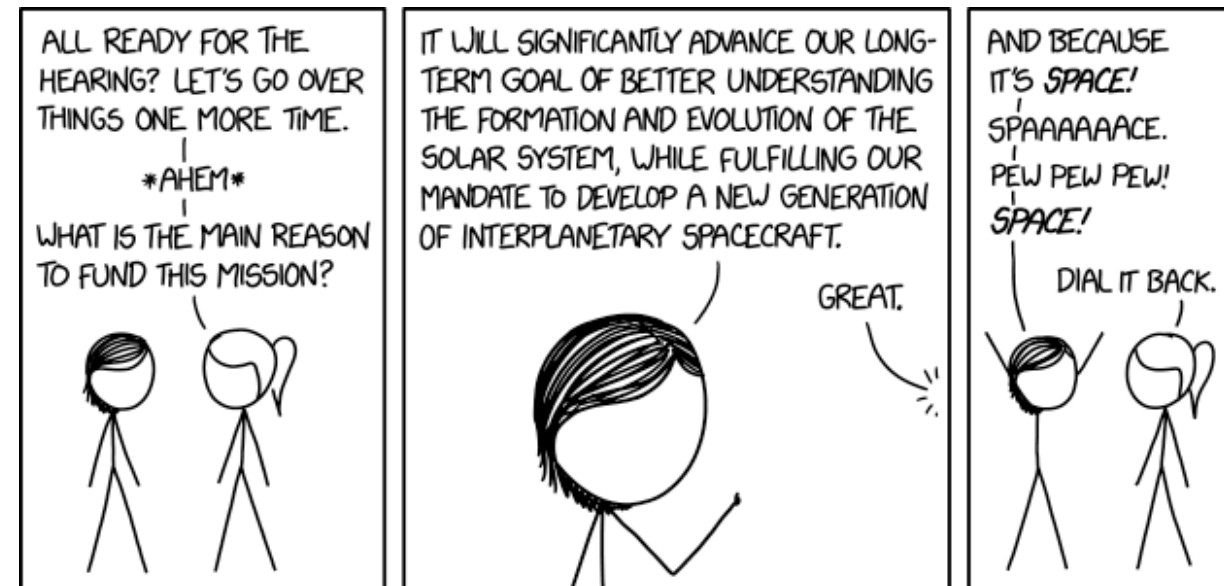
- Prelab discussion
- Determine improved IPC variant and design mutation primers
- Quiz beginning at 3:15pm

**\*\* 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 Wednesday 5/19

## • Mini-report (5%)

- completed as a team and submitted via Stellar
- due 5/14 at 10p

## • Quiz (collectively 10%)

- M3D4 (today!)

## • Notebook (part of 10% Homework and Notebook)

- due 5/13 at 10p M3D3

## • Blog (part of 5% Participation)

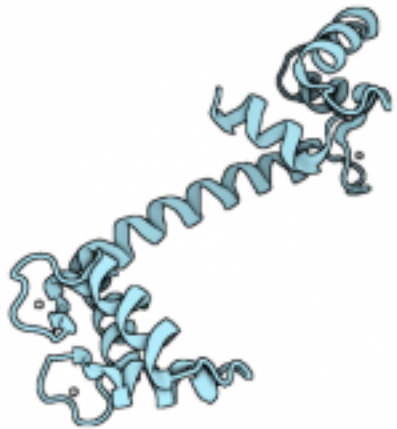
- due 5/20 at 10p via Slack (unless you have already completed 3 posts)

# Mod 3 Overview

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

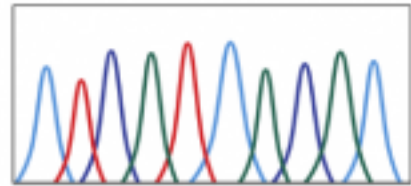
design

IPC

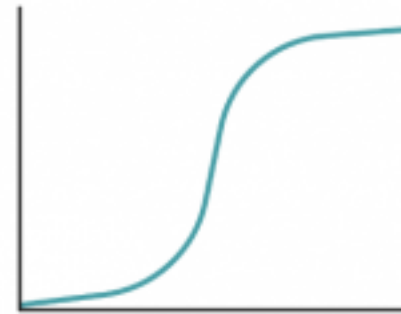


Day 1:  
examine sequence and  
structural features of IPC

modified  
protein



Day 2:  
identify mutations in  
variant IPC



Day 3:  
examine effects of mutations  
in variant IPC

Ca<sup>2+</sup> binding  
affinity

take away?



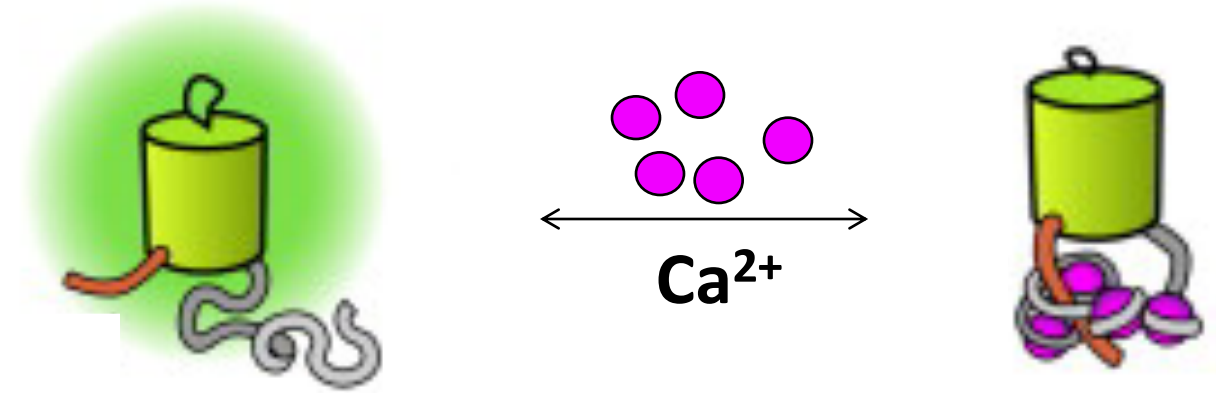
Day 4:  
design new variant IPC

↓  
design your  
own variant

# 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

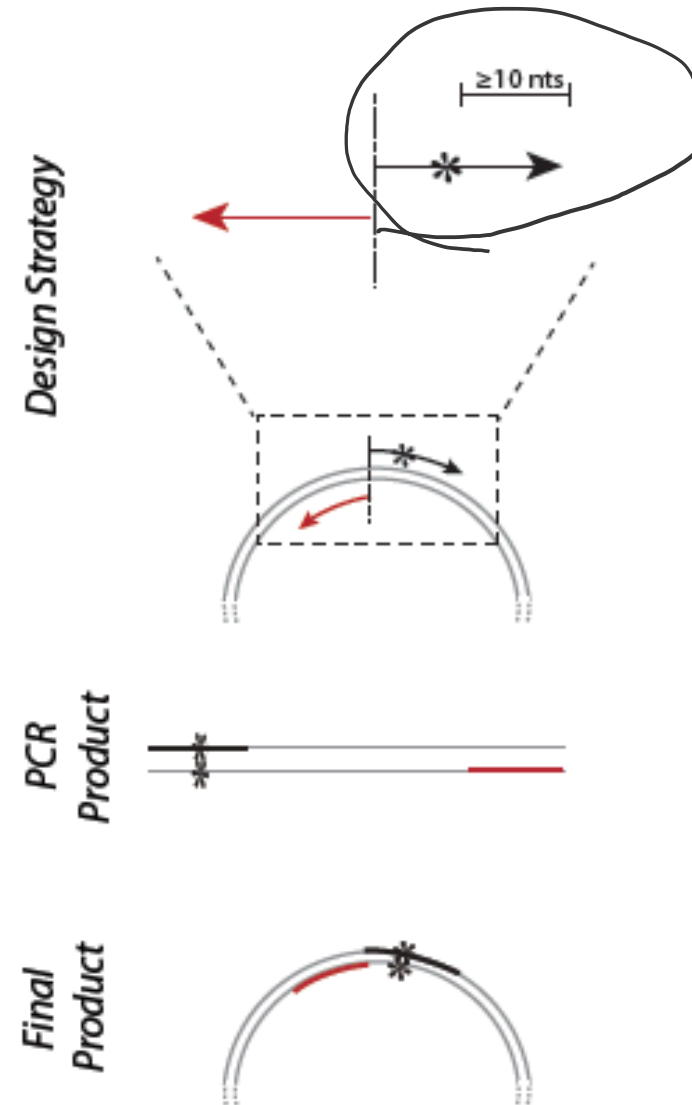
point mutation  
insertions  
deletions

- Forward primer: includes DNA modification

- PCR product: linear

- Final product: circular

on next slide

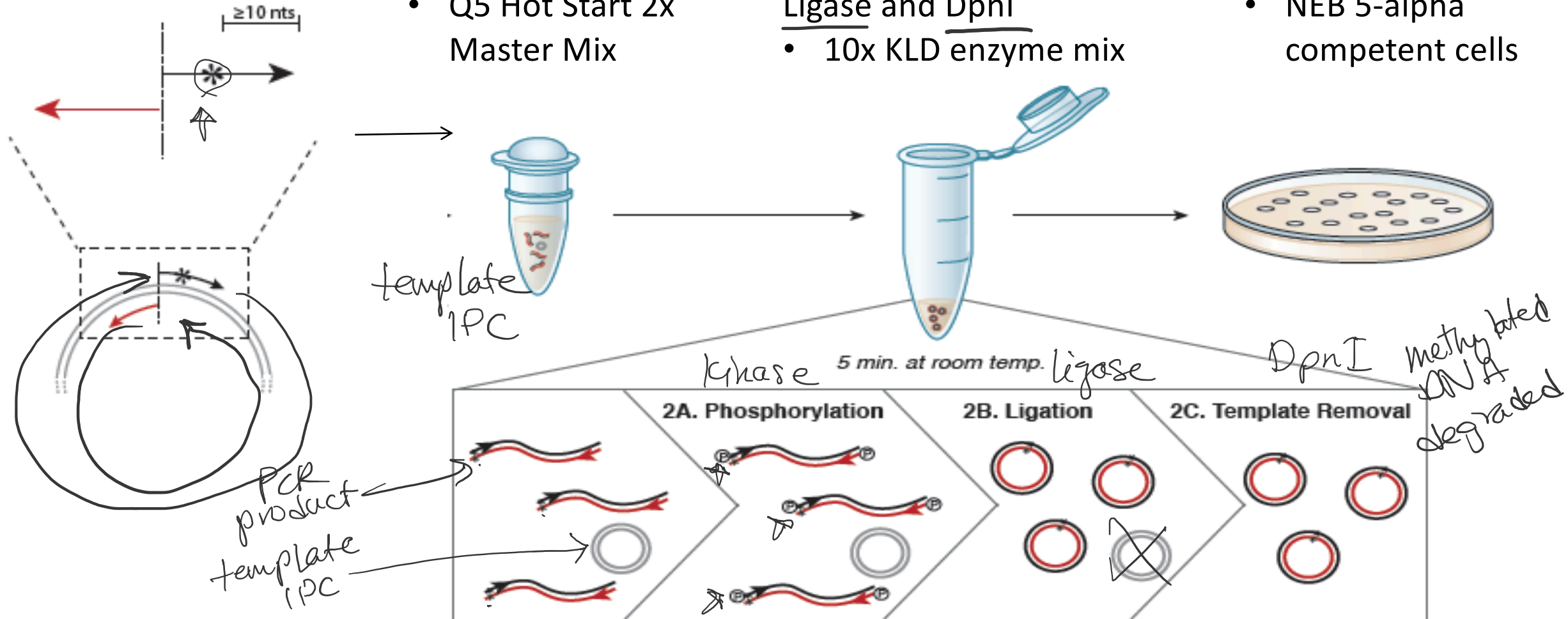


# SDM Part 2: Recover circular plasmid product using Kinase-Ligase-Dpn1 (KLD) enzyme mix

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



# For Today:

- Design optimized primer to alter calmodulin binding to calcium
- Finish lab notebooks (specifically M3D3 due 5/13 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

figures separate from text

- Audience has high familiarity with your project

- What figures will you include?

→ seq alignments, type of mutations of variants  
→  $\text{Ca}^{2+}$  titration binding curves  
→  $K_d$  values in table



For ~~Thursday~~  
FRIDAY



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FRIDAY

- ~~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 Thursday May 20 during lecture (11am)

