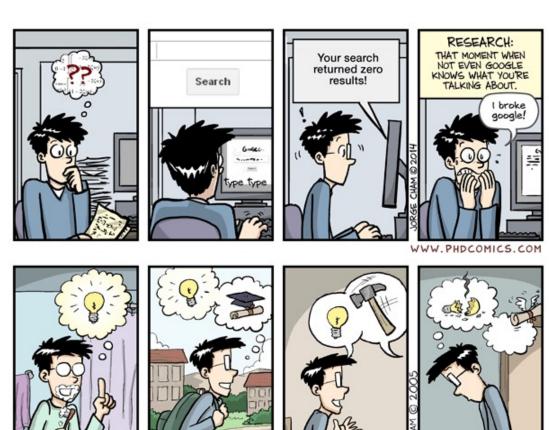
M3D3: Evaluate effect of mutation on IPC variants

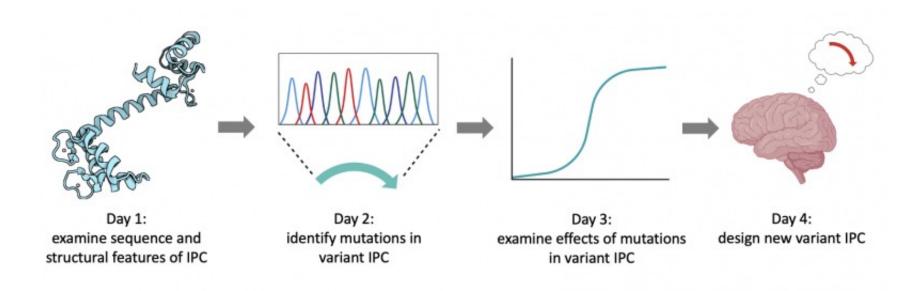
- 1. Comm Lab
- 2. Prelab discussion
- 3. Examine binding of IPC mutants
- 4. Work on research proposals!



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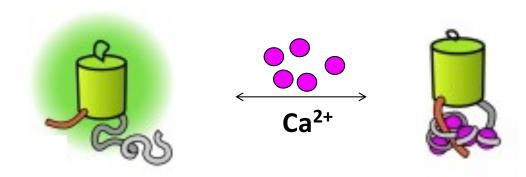
Mod 3 Overview

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

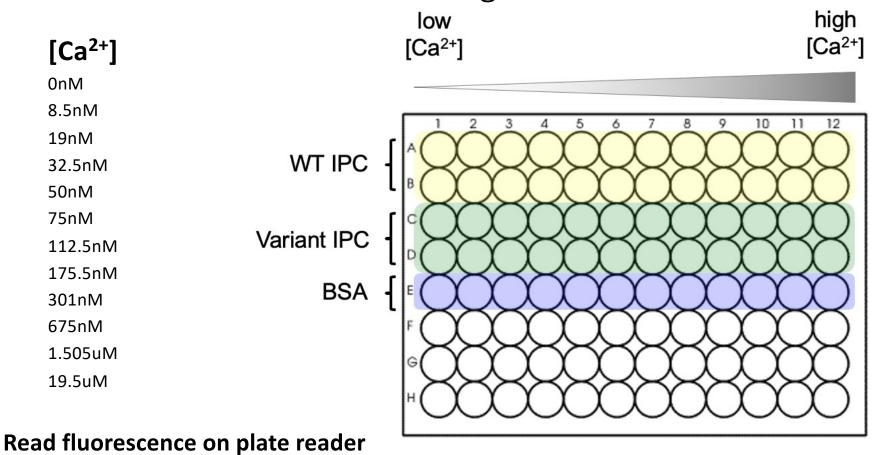


How will we evaluate our mutant IPC?

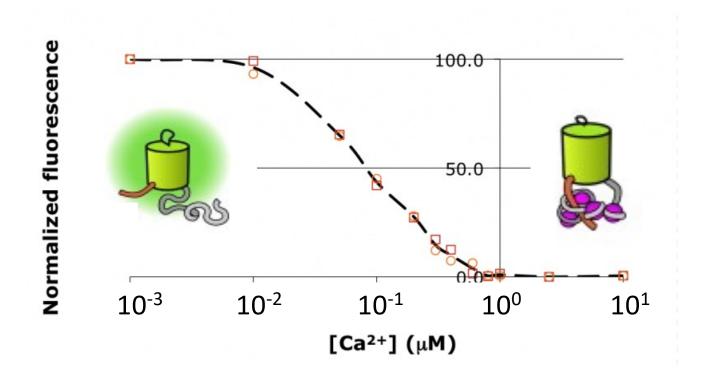
- Use solutions of known [Ca²⁺] calcium concentration
- Measure binding via fluorescence signal
- Generate titration curves
- Estimate/calculate K_d based on those curves



Calcium titration on 96-well plate used to generate IPC binding data

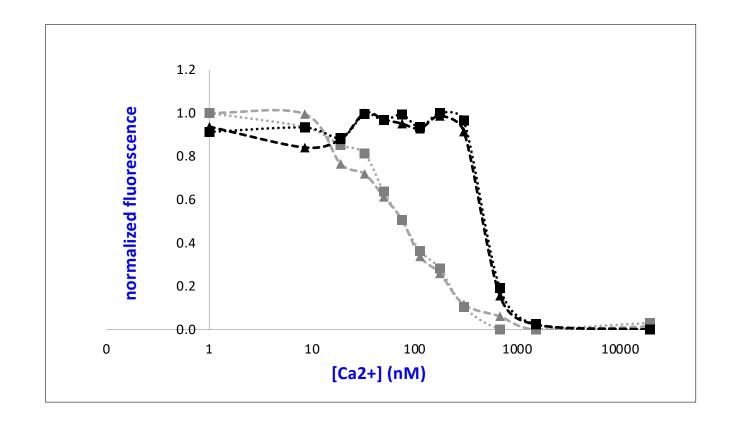


How do your mutations alter IPC-Ca²⁺ binding?

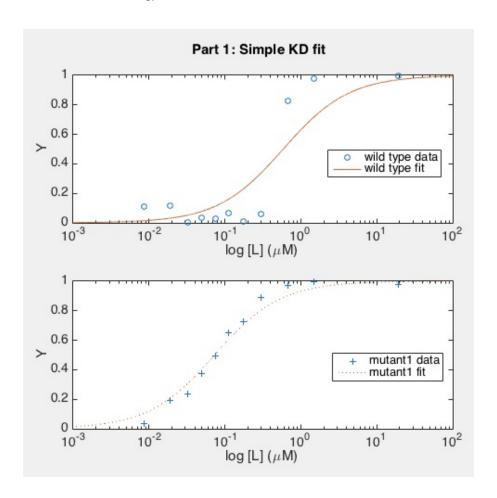


Plot your IPC-calcium titration data in Excel and estimate Kd

- Average duplicates
- Normalize to 0nM Ca²⁺



Fit apparent K_d in MATLAB



KD1 wt =
$$0.5858 \mu M$$

$$KD1 m1 = 0.0729 \mu M$$

- How good is the fit?
 - for wt-IPC?
 - for mutant?
- Does the calculated apparent K_d align with the estimated K_d?

For Today

- Work through the wiki to general IPC data set and calculate K_ds
- Work on Research Proposal!!!

For Thursday

- During Lecture: Give 2 minute pitch of research proposal idea to class
 - No slides
- On your own: Peer review of proposals and determination of societal and ethical impact within group

For M3D4 (5/12)

- Use feedback from proposal pitches to outline more complete written proposal
- Use wiki prompts in homework tab to guide outline