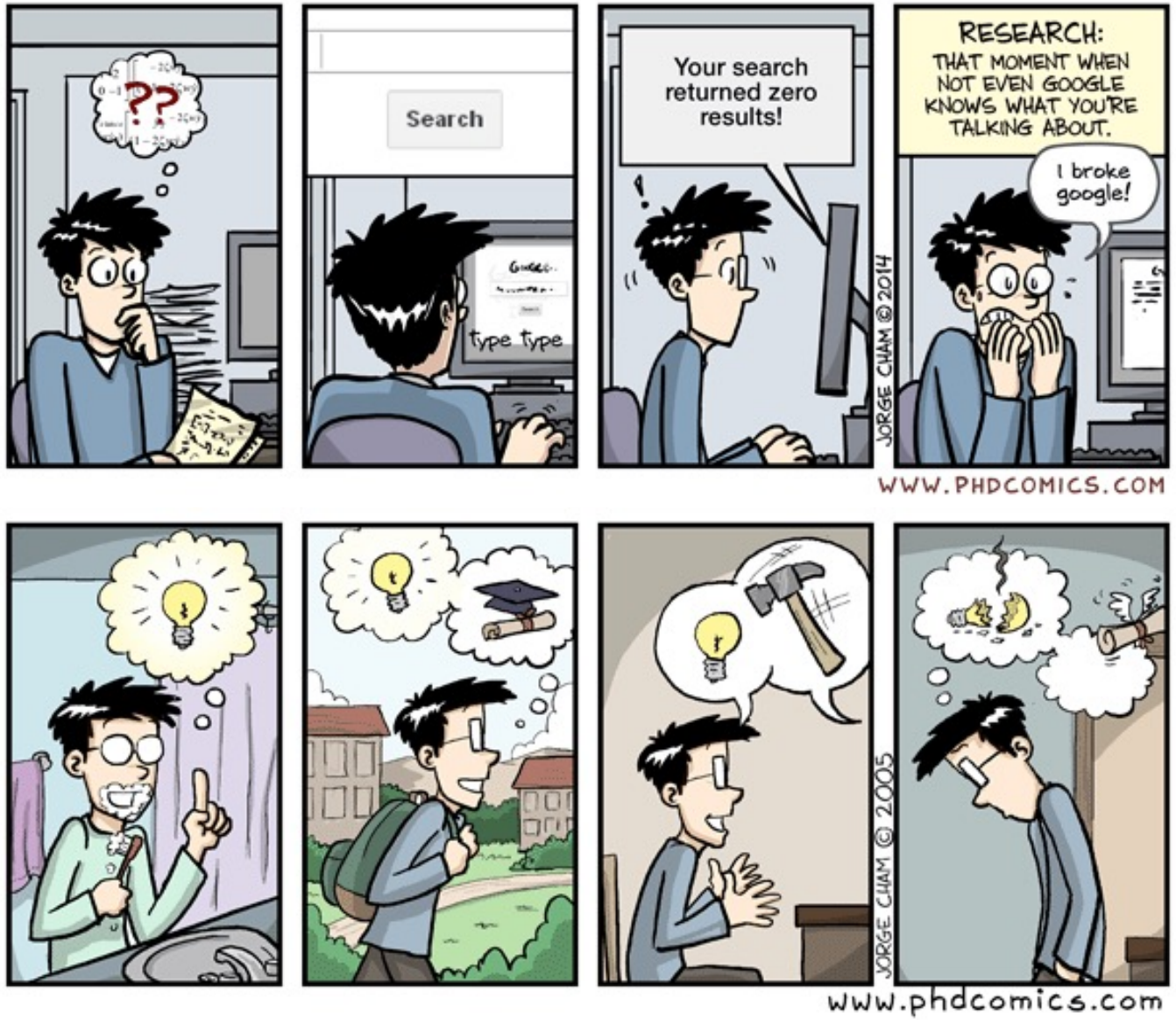
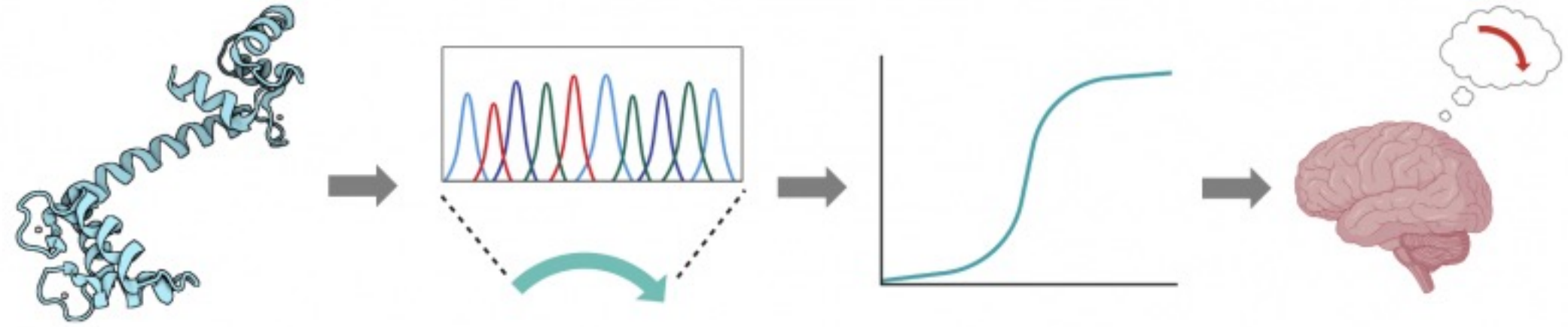
M3D3: Evaluate effect of mutation on IPC variants

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



Mod 3 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

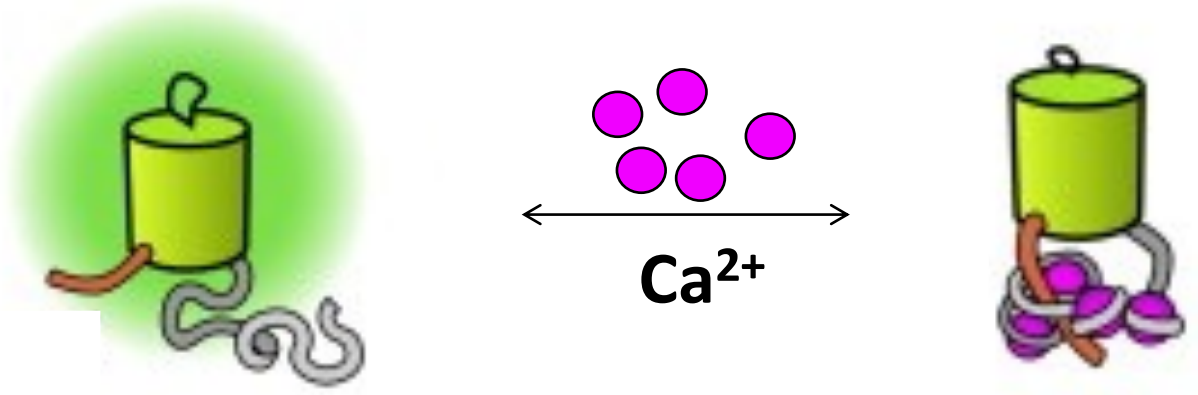
Day 2:
identify mutations in variant IPC

Day 3:
examine effects of mutations in variant IPC

Day 4:
design new variant IPC

How will we evaluate our mutant IPC?

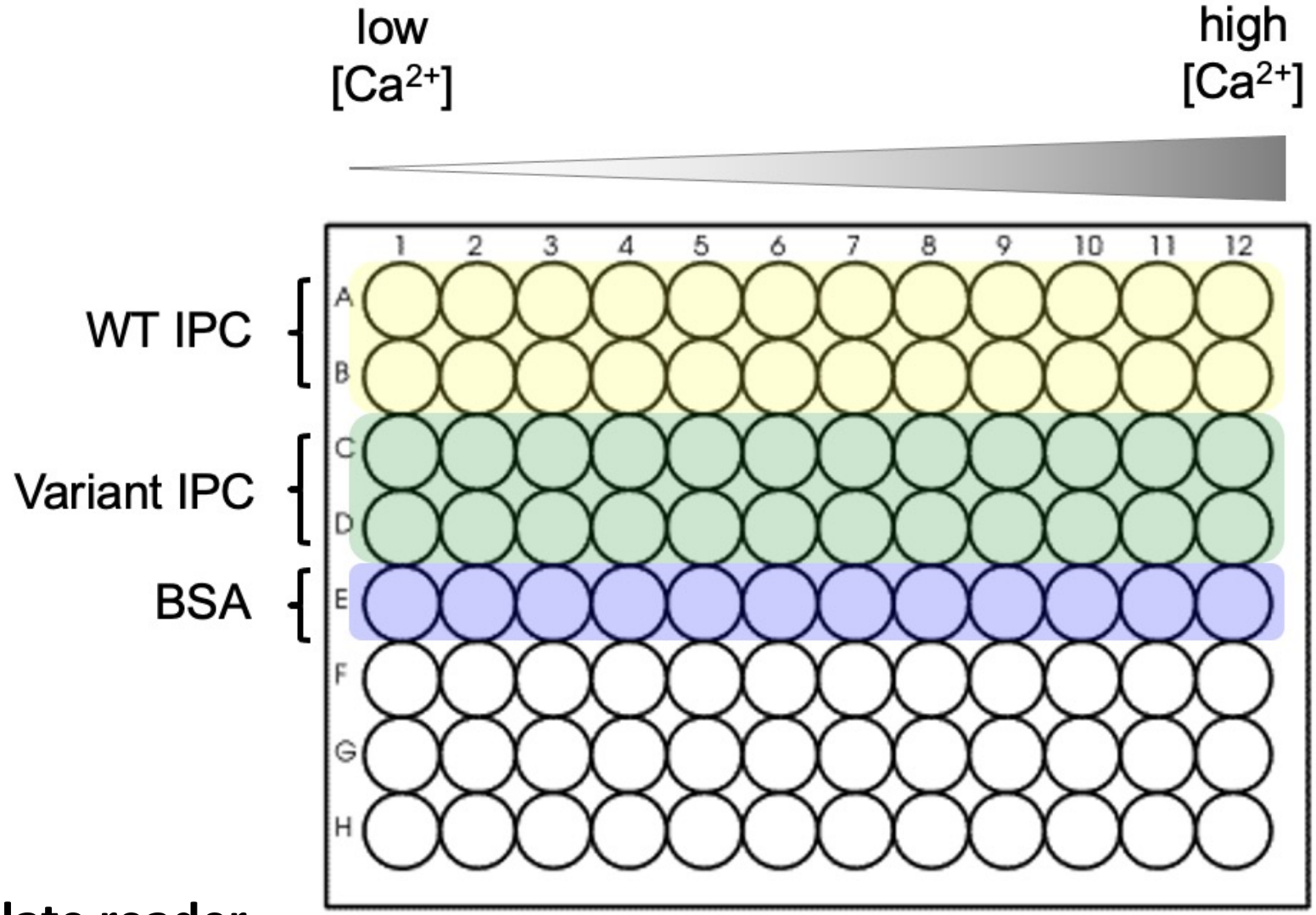
- Use solutions of known [Ca^{2+}] calcium concentration
- Measure binding via fluorescence signal
- Generate titration curves
- Estimate/calculate K_d based on those curves



Calcium titration plate used to generate binding data

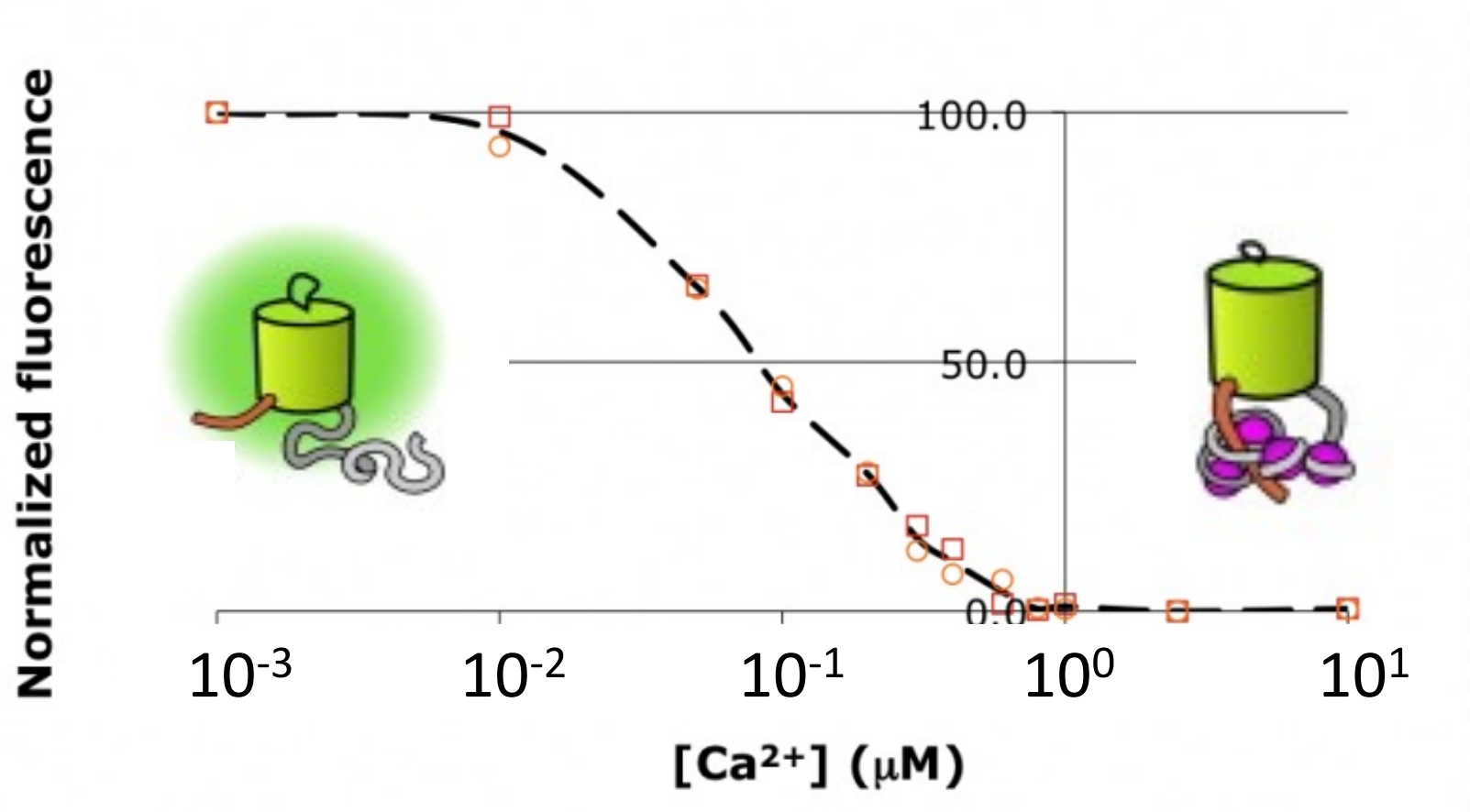
[Ca^{2+}]

0nM
8.5nM
19nM
32.5nM
50nM
75nM
112.5nM
175.5nM
301nM
675nM
1.505uM
19.5uM



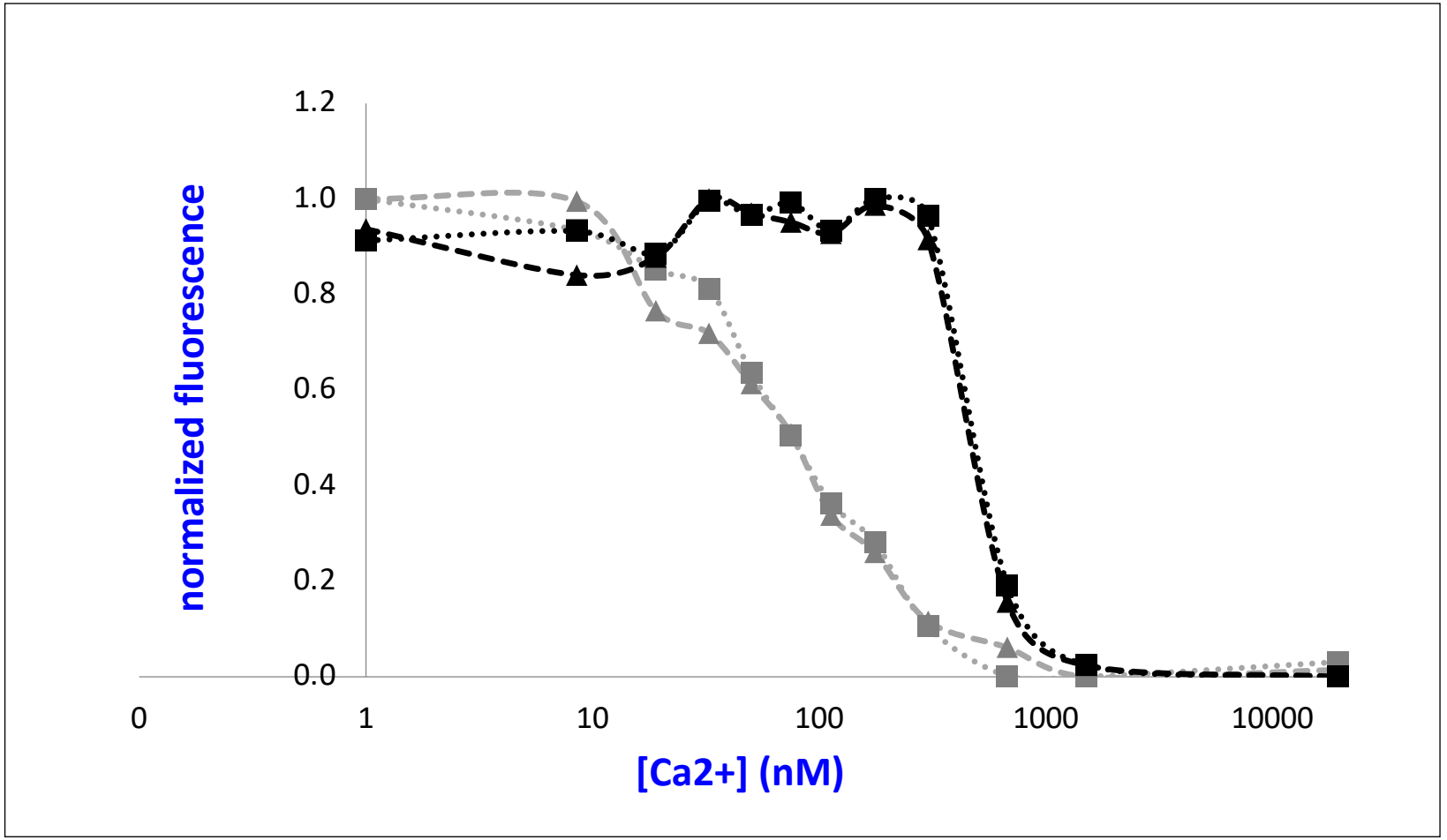
Read fluorescence on plate reader

How does your mutation alter IPC-Ca^{2+} binding?

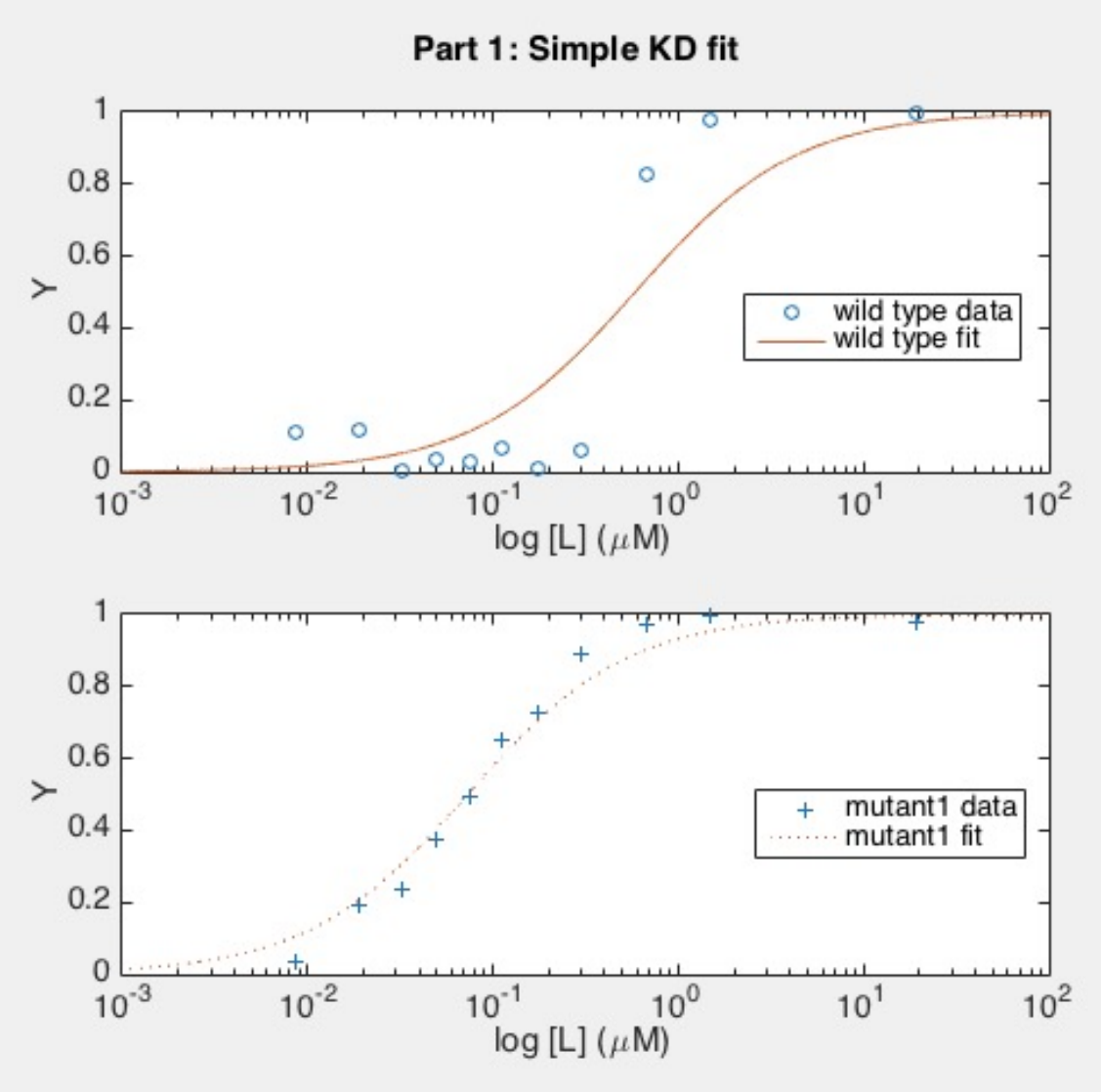


Plot your IPC-calcium titration data in Excel and estimate Kd

- Average duplicates
- Normalize to 0nM Ca^{2+}



Fit apparent K_d in MATLAB

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KD1_wt = 0.5858 μM

KD1_m1 = 0.0729 μM
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- 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
- During Lab: Peer review of proposals and determination of societal and ethical impact within group

For M3D4 (5/11)

- Use feedback from proposal pitches to write up more complete written proposal
- Use wiki prompts