

M2D6: Characterize protein

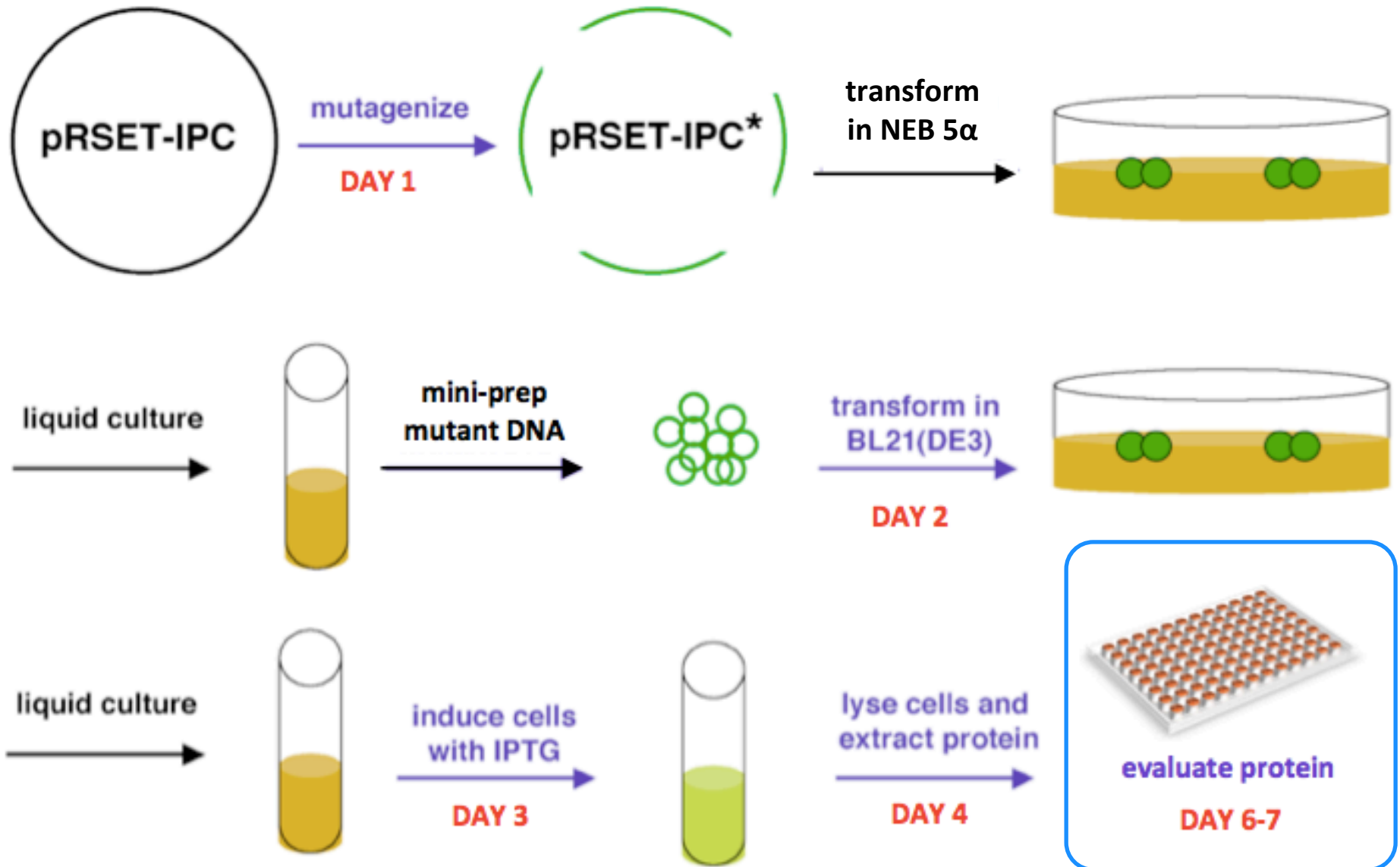
10/29/2015

M2 is coming to an end...

- due M2D7:
 - comment on your peer's methods
- on M2D7:
 - quiz
 - lab notebook graded
 - Excel and Matlab
- recounting M2:
 - protein engineering report
due at 5pm on Saturday, November 14
 - blog post [you can write about your journal club experience!](#)
due at 5pm on Sunday, November 15

[4 blogs needed total this semester](#)

Last day of experiments for Module 2!

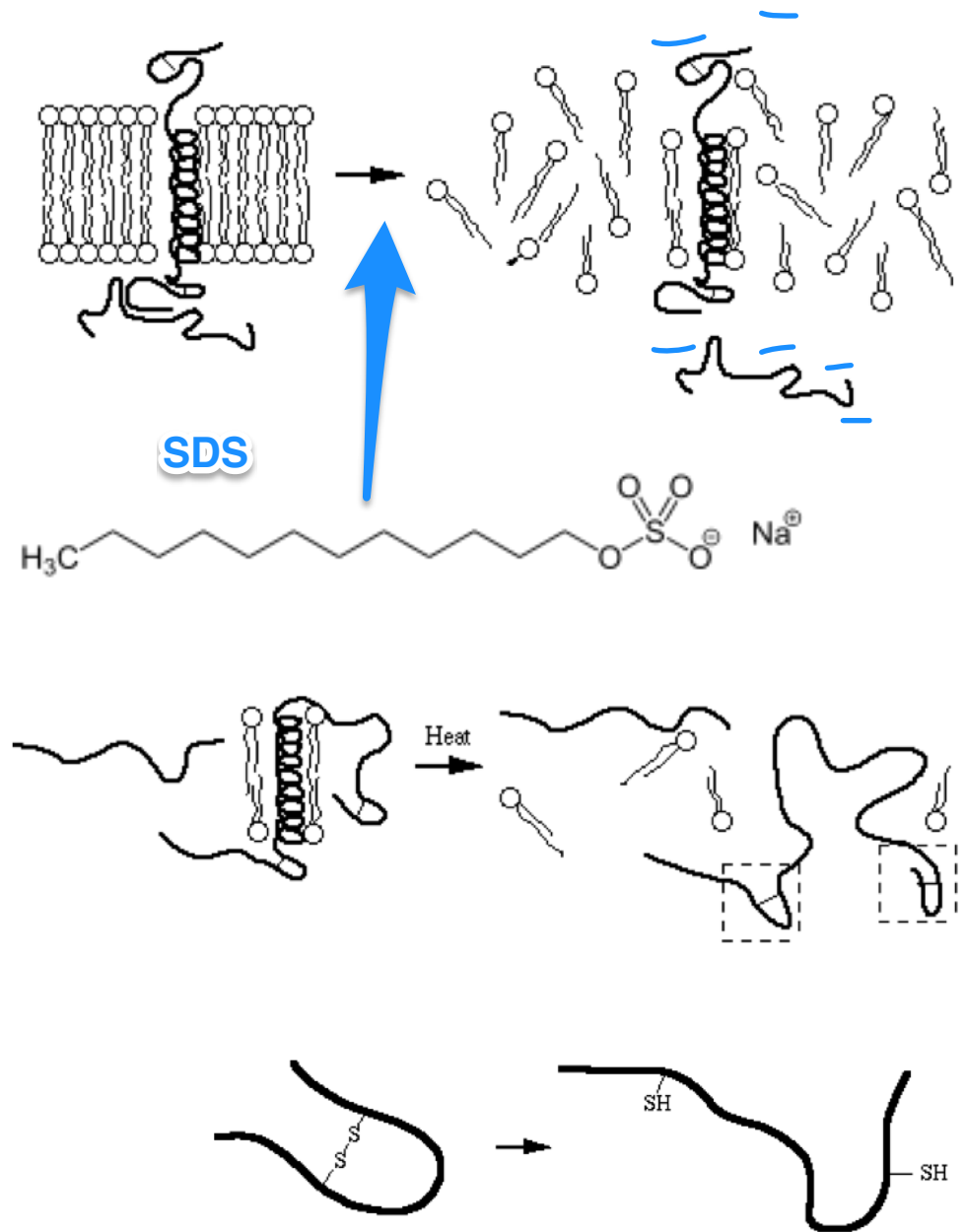


SDS-PAGE separates proteins by size

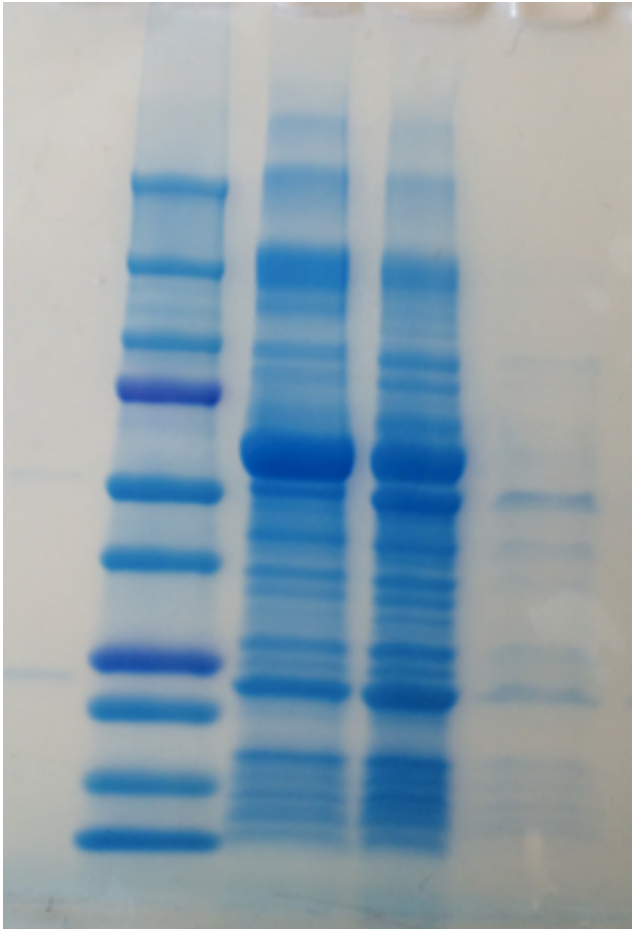
- Laemmli buffer:

- SDS
- glycerol
- beta-mercaptoethanol
- bromophenol blue
- Tris-HCl

- boiling



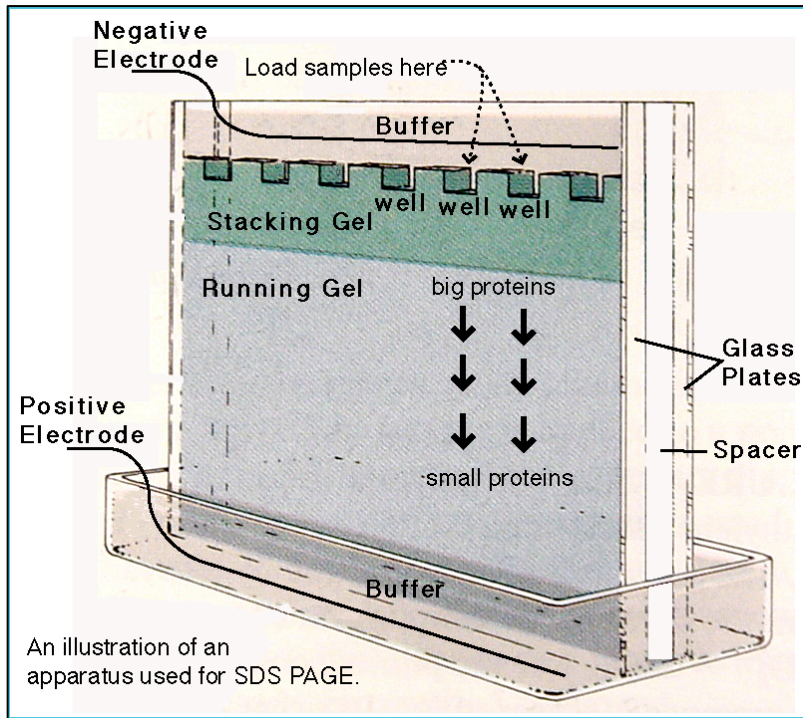
Load 6 samples + 2 ladders on SDS-PAGE gel



- Loading order:
 - think about figure(s) in your Results
 - wild-type IPC cell lysate – IPTG / + IPTG
 - X#Z mutant IPC cell lysate – IPTG / + IPTG
 - purified wt and mutant IPC
 - stained and unstained ladders

track progress of gel, estimate gross protein amounts
- 4-15% acrylamide gel:
 - for 10-250 kDa proteins
 - inverse pericam:
 - 1281** bp = **427** a.a. = **47** kDa
 - ~ 110 Da / a.a
 - His-tag ~ 3 kDa

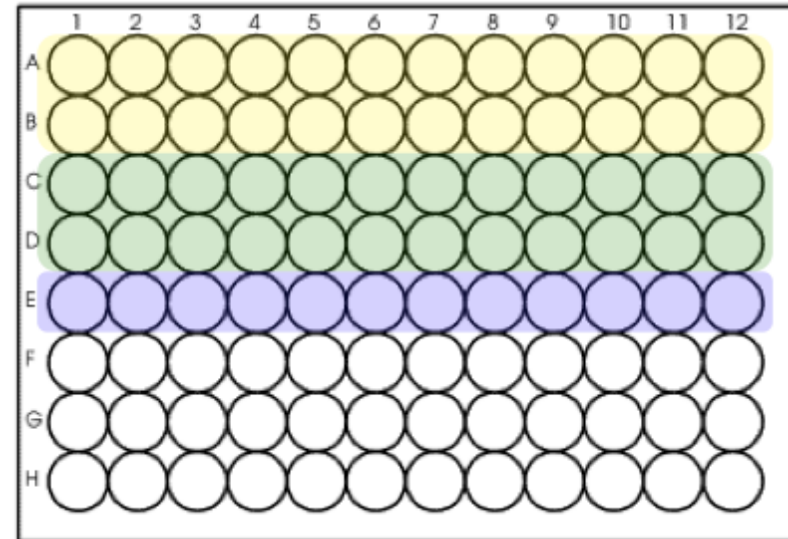
expect a band for IPC at 50 kDa



- **SDS-PAGE**

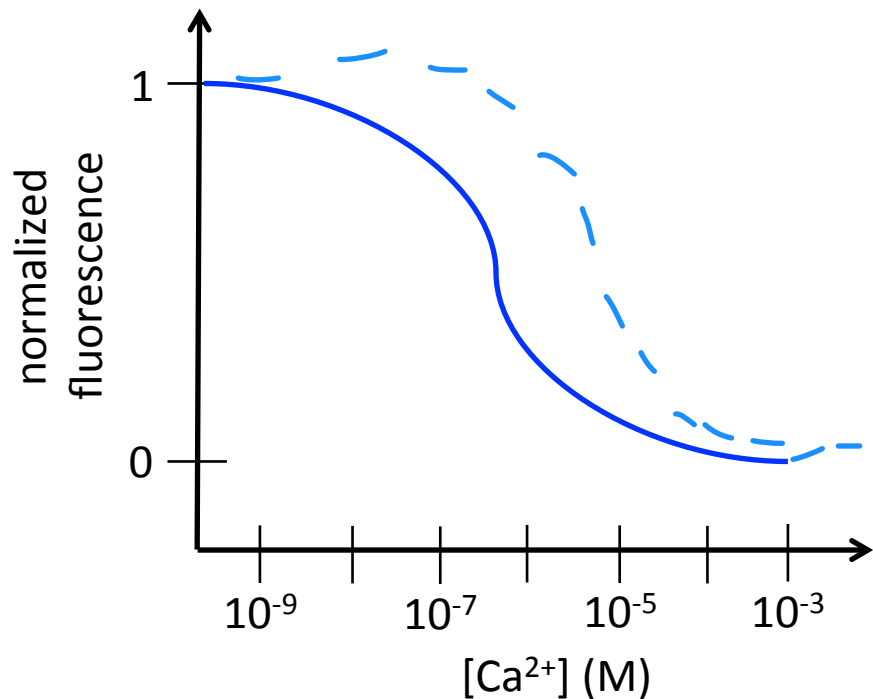
- boil samples
- load in lanes 2-9
- run at 200 V for 30 min
- rinse with water
- stain with Coomassie

Today in lab



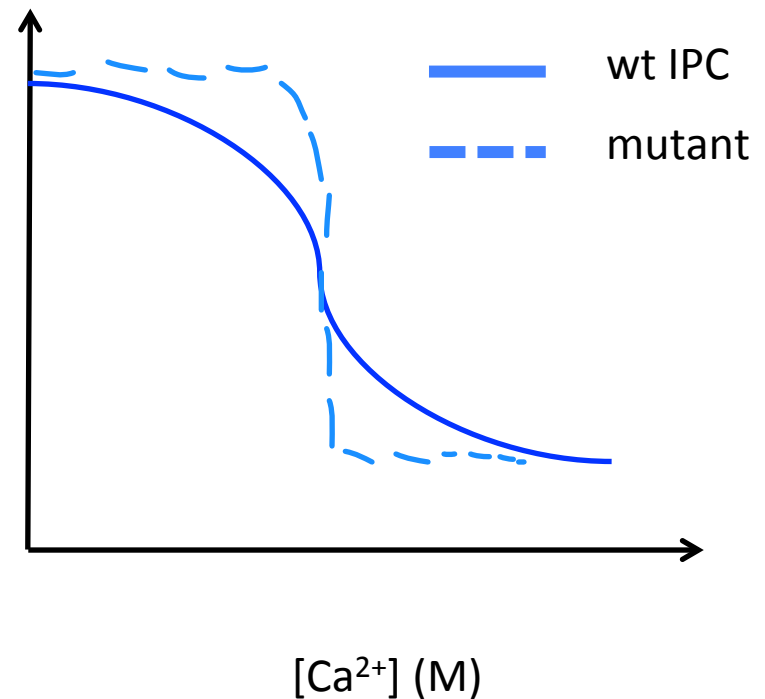
- **IPC-calcium titration**
 - prepare 96-well plate
 - read fluorescence levels with plate reader

Protein engineering: Did your mutation affect IPC binding affinity and/or cooperativity



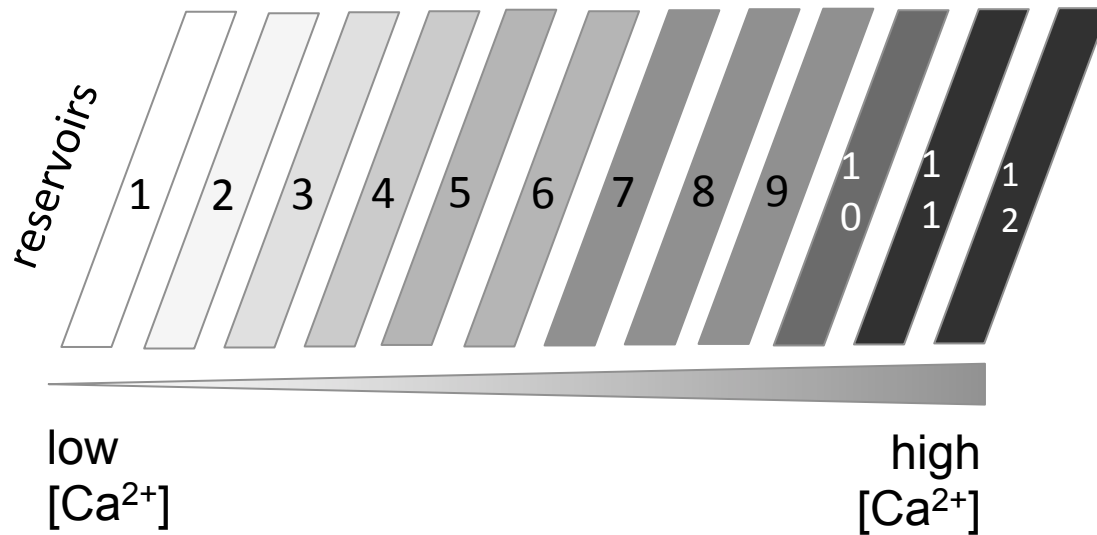
- (only) affinity changes

mutant has lower affinity with Ca^{2+}

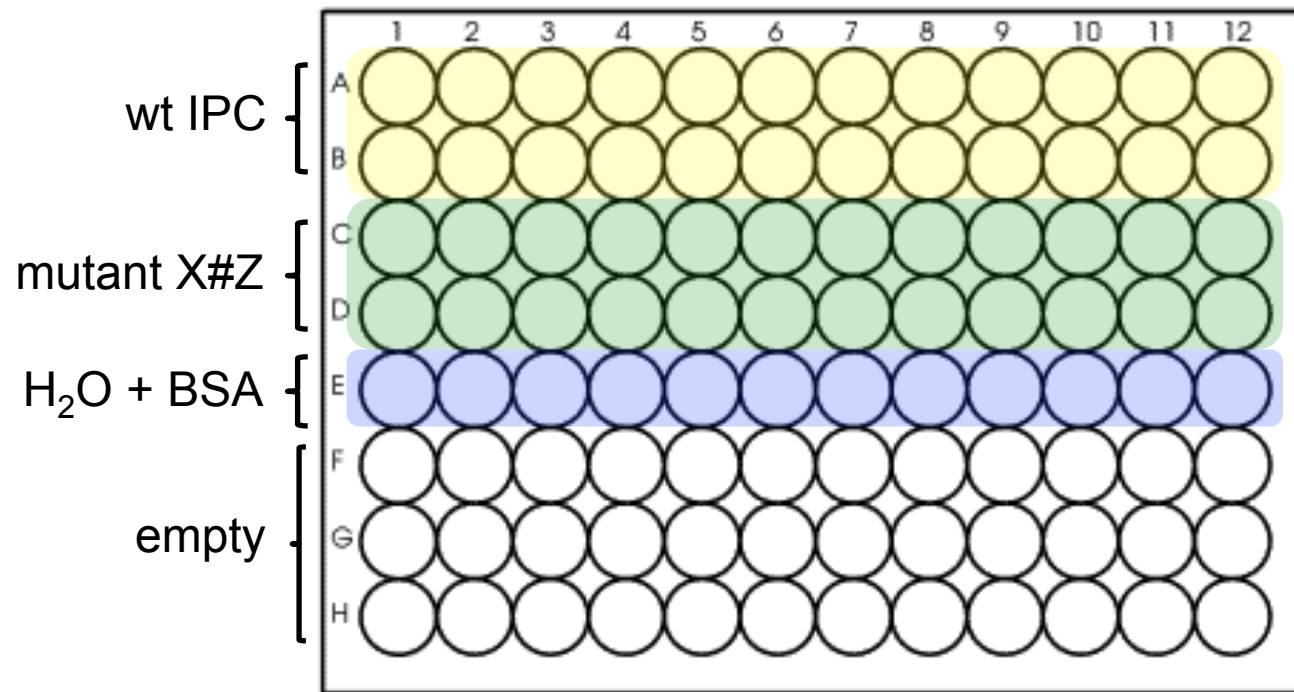


- (only) cooperativity changes

mutant shows higher cooperativity in binding the 4 Ca^{2+}

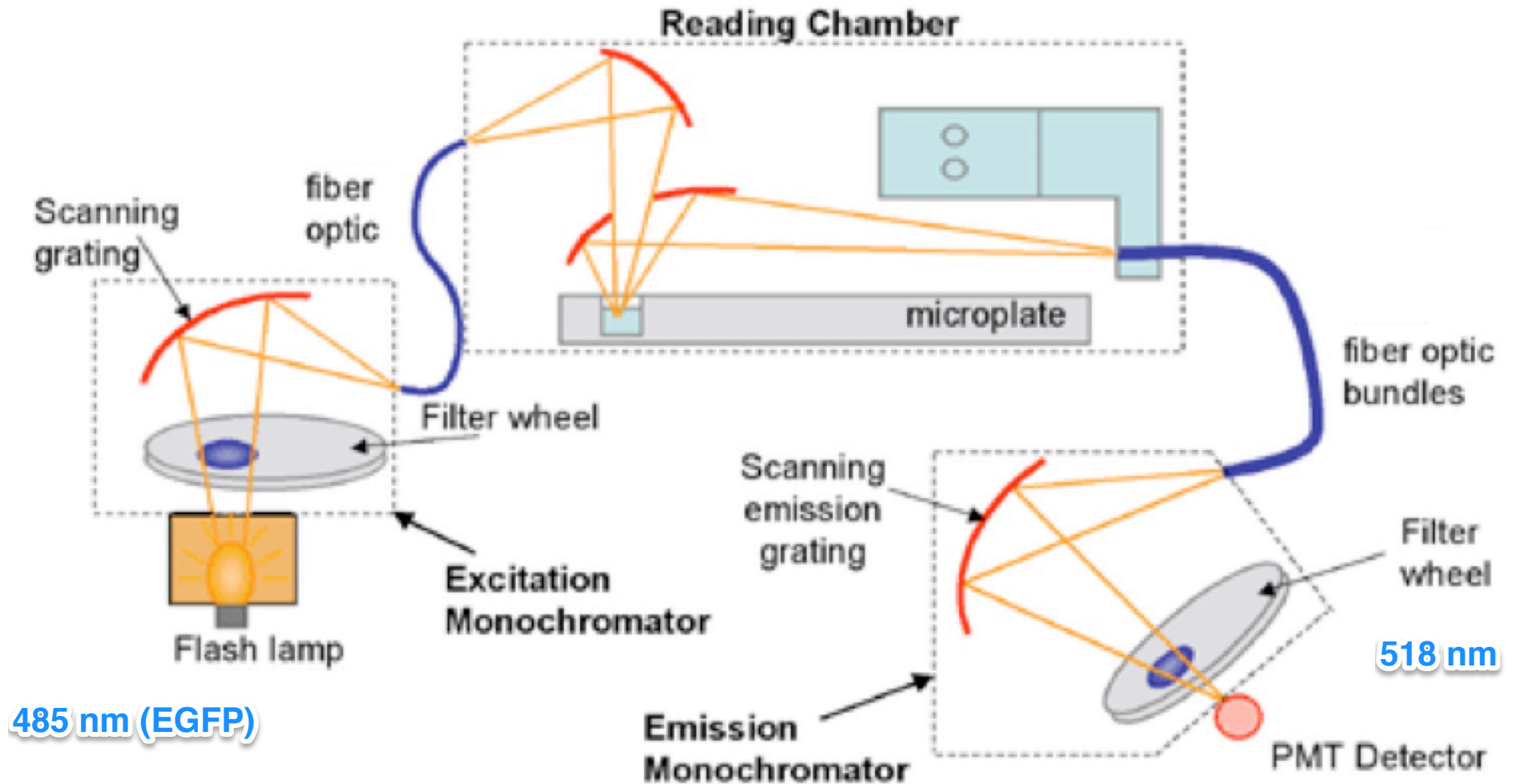


known concentrations
from 0 nM, 8.5 nM, ...,
675 nM, ..., 19.5 μ M



30 μ L IPC
30 μ L calcium solution
in each well

Fluorescence plate reader



Assay inverse pericam

- Excitation: 485 nm
- Emission: 518 nm

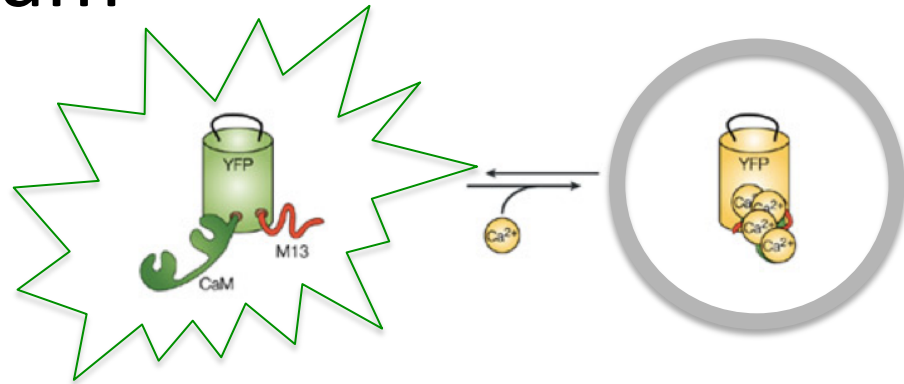
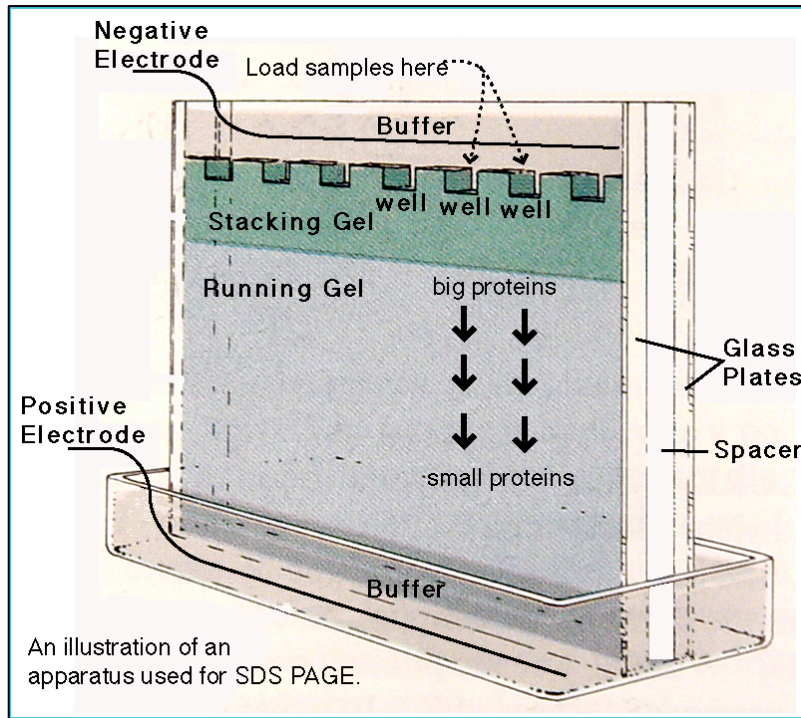


plate													
D132H	0.926	0.960	0.985	0.965	1.038	0.780	0.987	1.028	0.923	0.323	0.286	0.256	
D132H	0.706	0.851	0.799	0.780	0.919	0.804	1.037	0.914	0.852	0.344	0.310	0.308	
wt IPC	0.528	0.443	0.430	0.398	0.359	0.331	0.316	0.263	0.239	0.166	0.175	0.178	
wt IPC	0.489	0.477	0.477	0.424	0.373	0.313	0.305	0.303	0.258	0.170	0.182	0.167	
water+BSA	0.015	0.014	0.015	0.017	0.011	0.013	0.010	0.013	0.016	0.013	0.012	0.011	
empty	0.014	0.015	0.010	0.010	0.011	0.017	0.015	0.010	0.015	0.011	0.016	0.013	
empty	0.014	0.011	0.017	0.175	0.015	0.016	0.011	0.011	0.010	0.012	0.009	0.013	
empty	0.011	0.011	0.012	0.012	0.014	0.012	0.012	0.011	0.017	0.016	0.013	0.008	

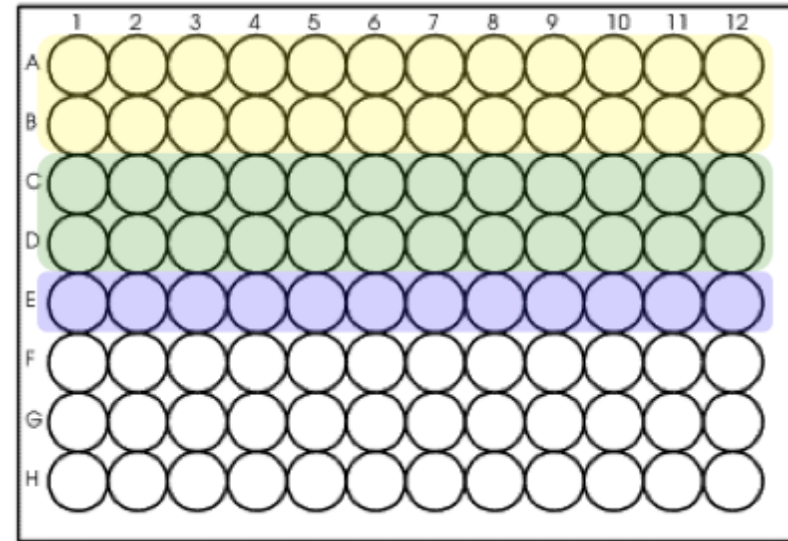
- To be analyzed on M2D7...
 - Excel
 - Matlab

Today in lab



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