

M2D6: Characterize protein

10/30/2015

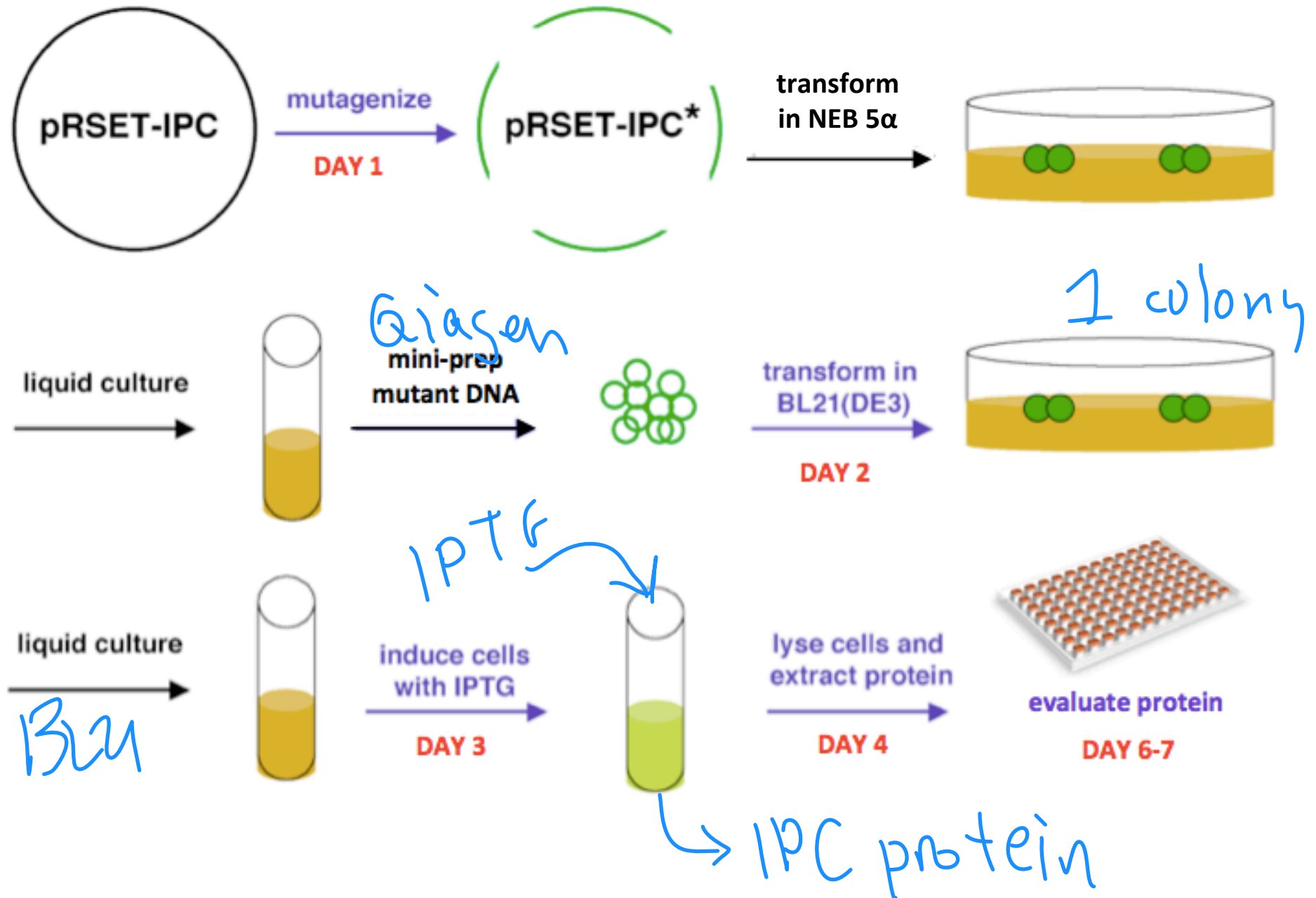
1. Prelab Discussion
2. Boil samples, load SDS-PAGE gels
3. Prepare samples for calcium titration curve
4. Measure IPC fluorescence on Fluorometer (Building 68)
5. Stain SDS-PAGE gel with coomassie

M2 is coming to an end...

- Homework due M2D7:
 - comment on your peer's methods
- on M2D7:
 - quiz
 - lab notebook graded
 - Excel and Matlab analysis
- Assessments for M2:
 - protein engineering report
due at 5pm on Saturday, November 14
 - blog post
due at 5pm on Sunday, November 15

→ JC blog
4 blogs total, up to 7 blog posts

Last day of experiments for Module 2!



SDS-PAGE separates proteins by size

6X Sample buffer

- Laemmli buffer:

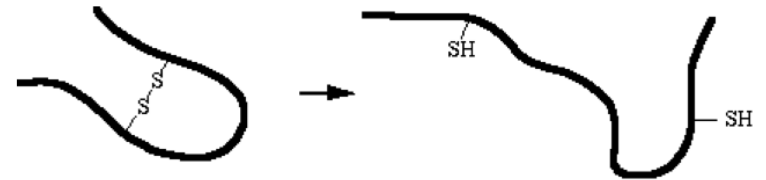
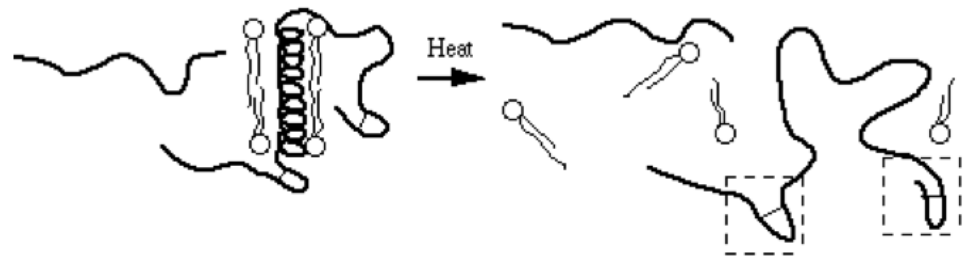
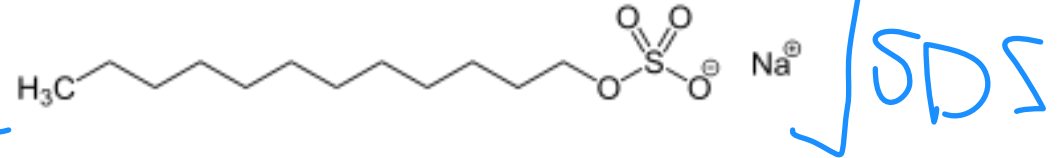
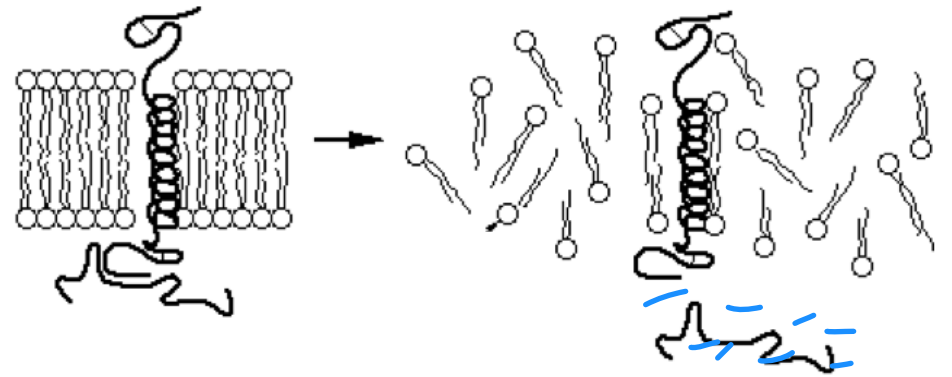
- SDS
- glycero

3-5kDa bromophenol blue

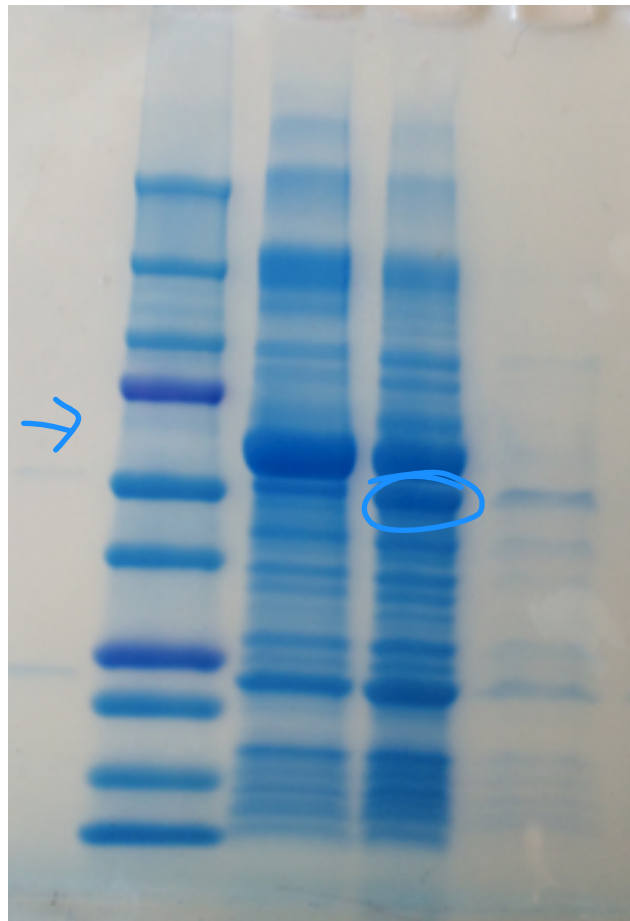
- β ME reducing
- Tris-HCl

- boiling

→ 5min



Load 6 samples + 2 ladders on SDS-PAGE gel



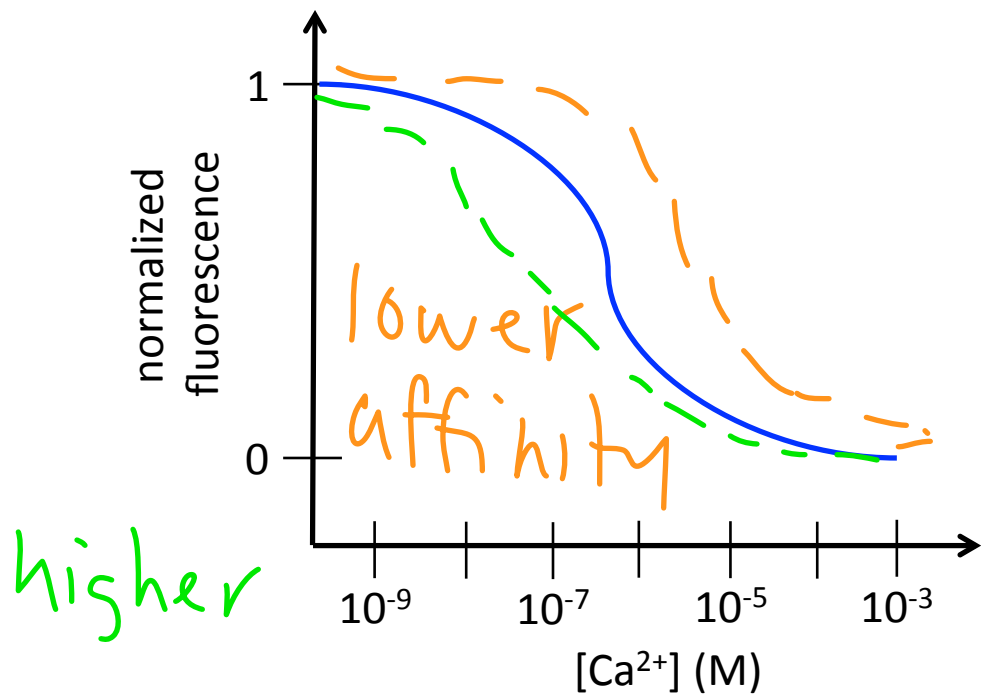
- Loading order:
 - think about figure(s) in your Results
 - wild-type IPC cell lysate - IPTG / + IPTG
 - X#Z mutant IPC cell lysate - IPTG / + IPTG
 - purified wt and mutant IPC
 - stained and unstained ladders = 10 μ l
- 4-15% acrylamide gel: *Amount*
 - for 10-250 kDa proteins
 - inverse pericam:
 - $1281 \text{ bp} = 427 \text{ a.a.} = 47 \text{ kDa}$
 - $\sim 110 \text{ Da / a.a.}$
 - His-tag $\sim 3 \text{ kDa}$
 - $\sim 50 \text{ kDa}$

blue \uparrow
pink

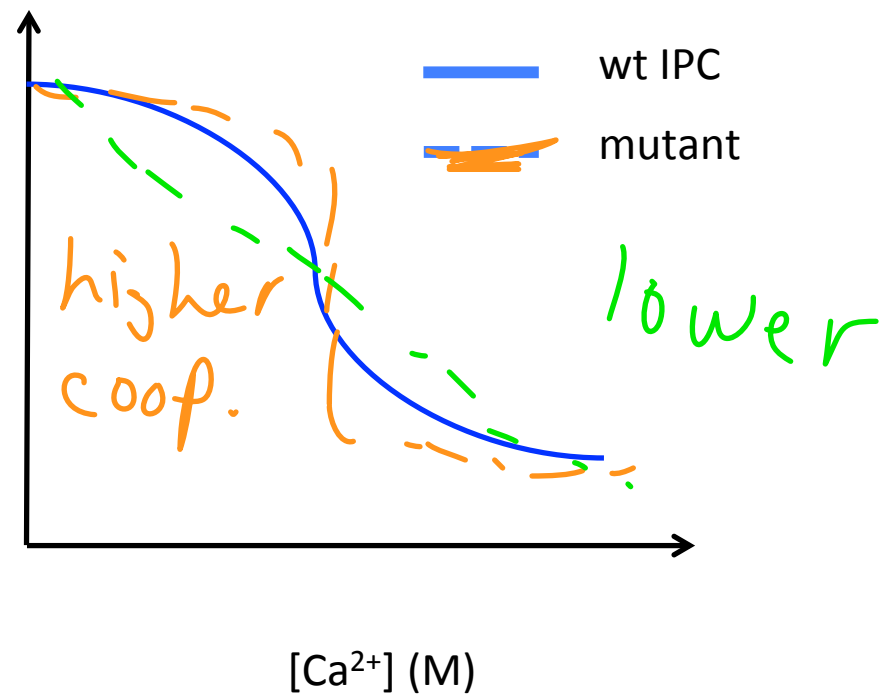
estimate protein

bp/3 = AA
AA x 110 = ~~110~~ a

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

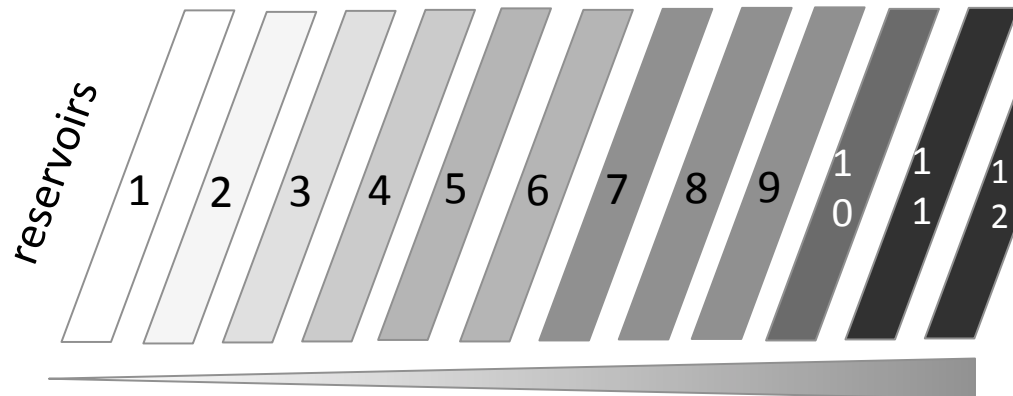


- (only) affinity changes



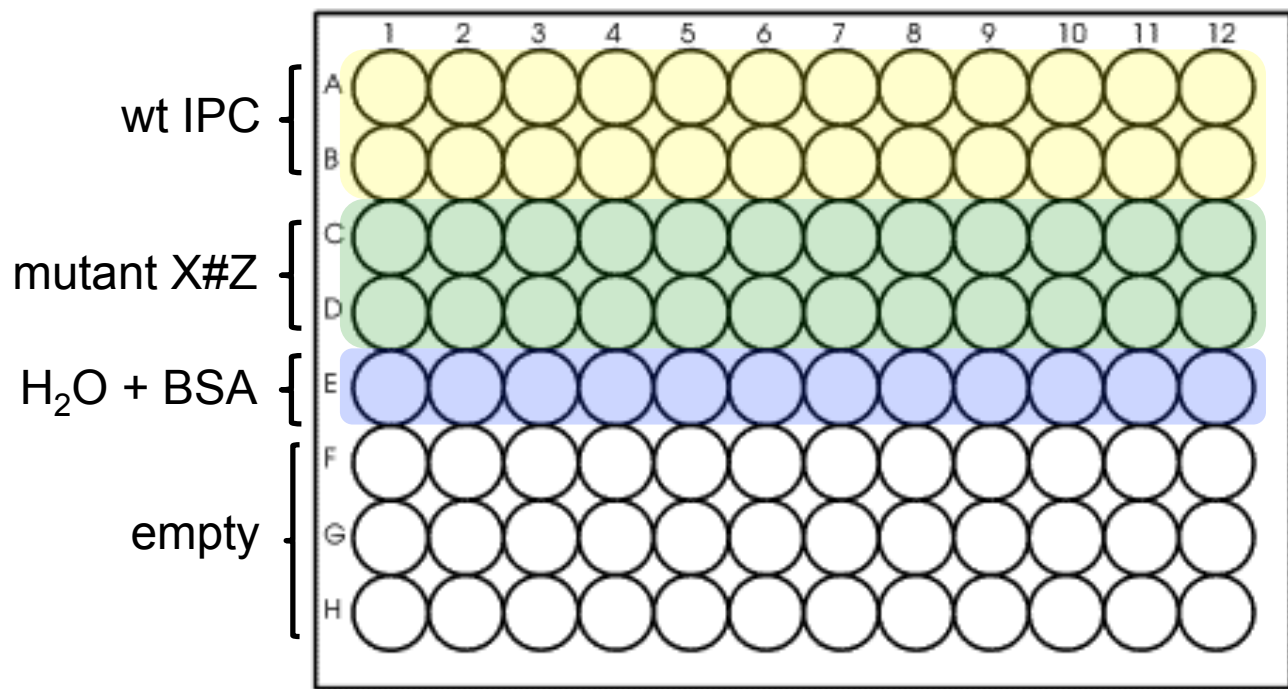
- (only) cooperativity changes

black ≠ crystal k



low
[Ca²⁺]

high
[Ca²⁺]



known concentrations
0nM, 25nM...
675nM .. 19.5µM



30ml calcium soln
30ml IPC

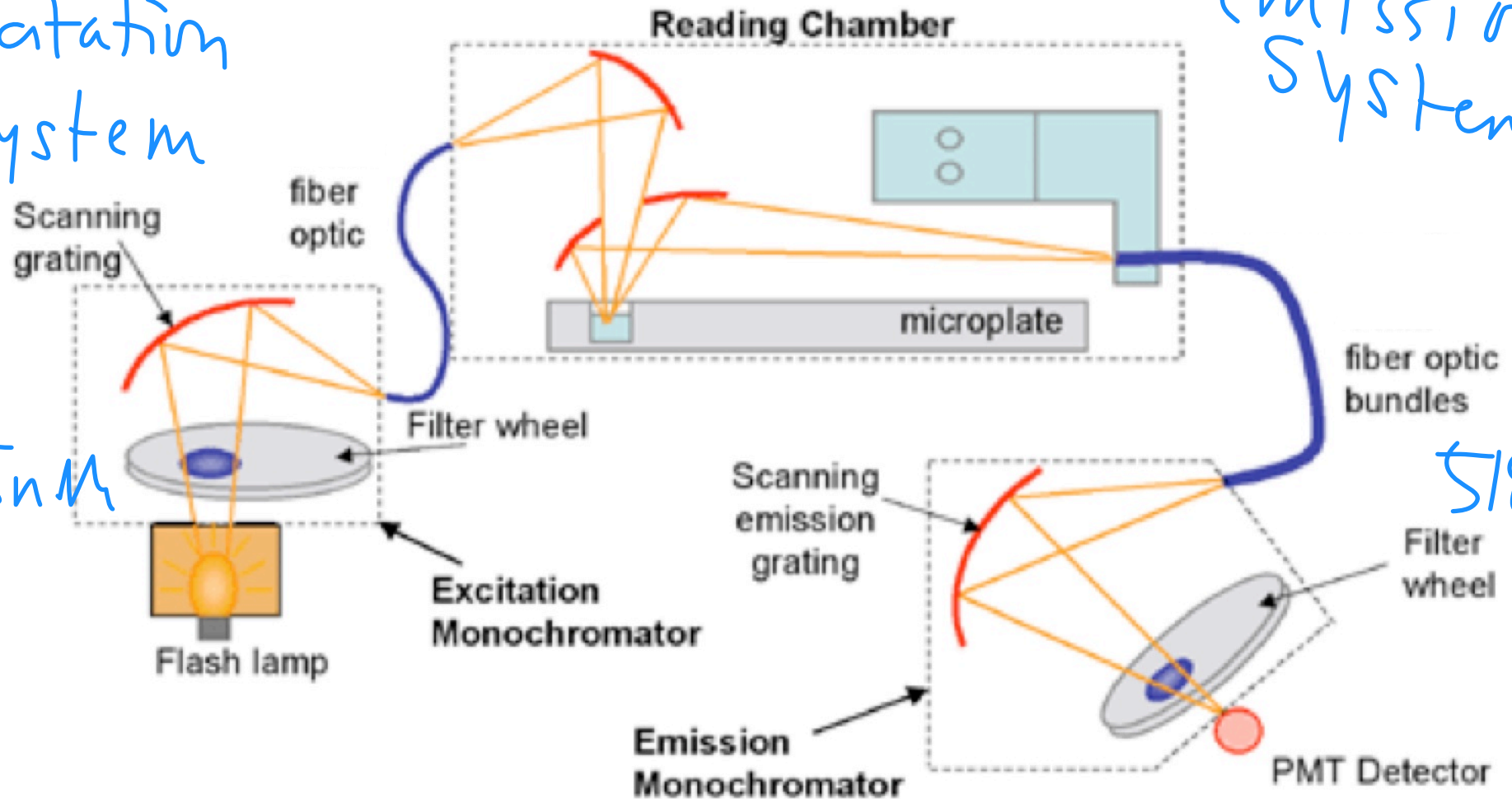
Fluorescence plate reader

excitation system

emission system

485nm

518nm



Assay inverse pericam

- Excitation: 485 nm
- Emission: 518 nm

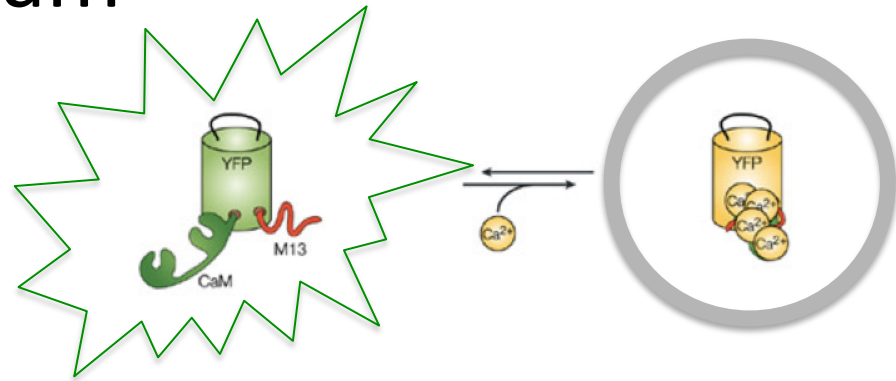
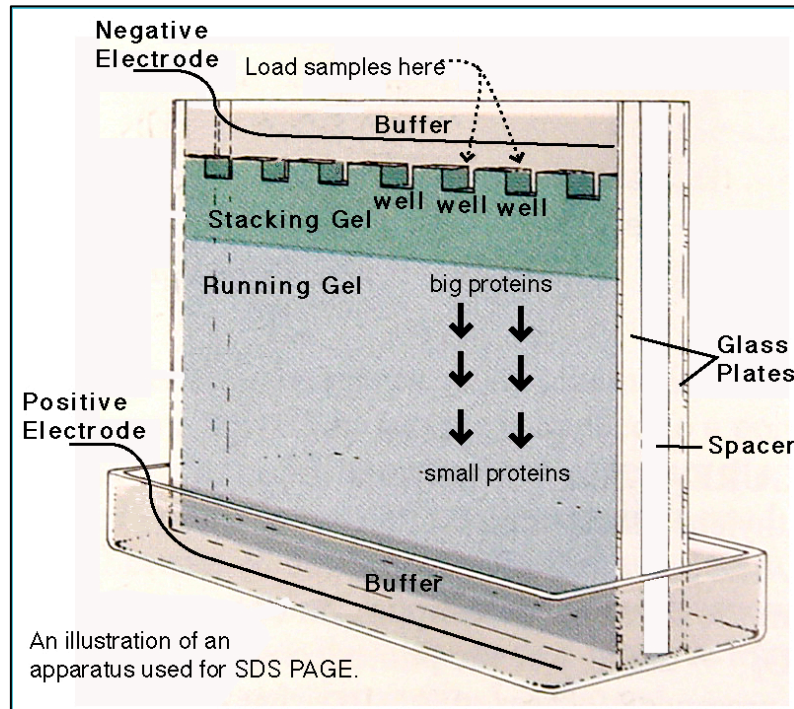


plate													
D132H	0.926	0.960	0.985	0.965	1.038	0.780	0.987	1.028	0.923	0.323	0.286	0.256	
D132H	0.706	0.851	0.799	0.780	0.919	0.804	1.037	0.914	0.852	0.344	0.310	0.308	
wt IPC	0.528	0.443	0.430	0.398	0.359	0.331	0.316	0.263	0.239	0.166	0.175	0.178	
wt IPC	0.489	0.477	0.477	0.424	0.373	0.313	0.305	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	

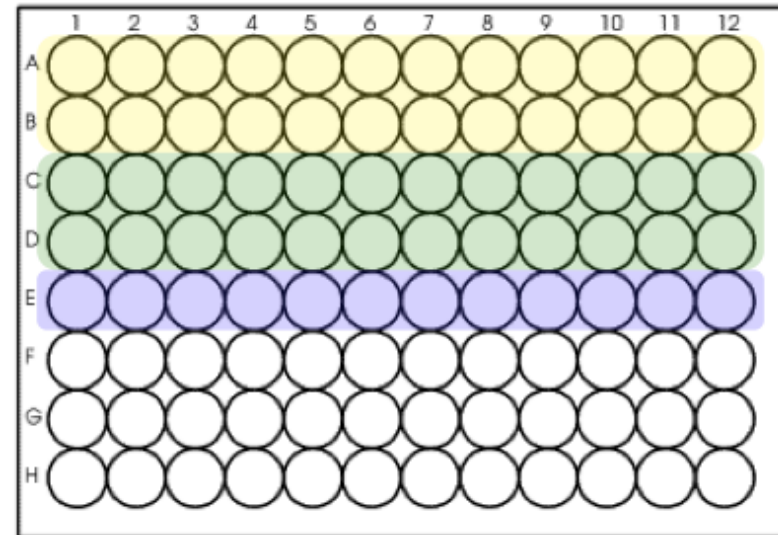
- To be analyzed on M2D7...
 - Excel
 - Matlab

Today in lab



- SDS-PAGE

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



- IPC-calcium titration

- prepare 96-well plate
- read fluorescence levels with plate reader