M1D7: Characterize protein

3/01/2016

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

MOD1 is coming to an end…

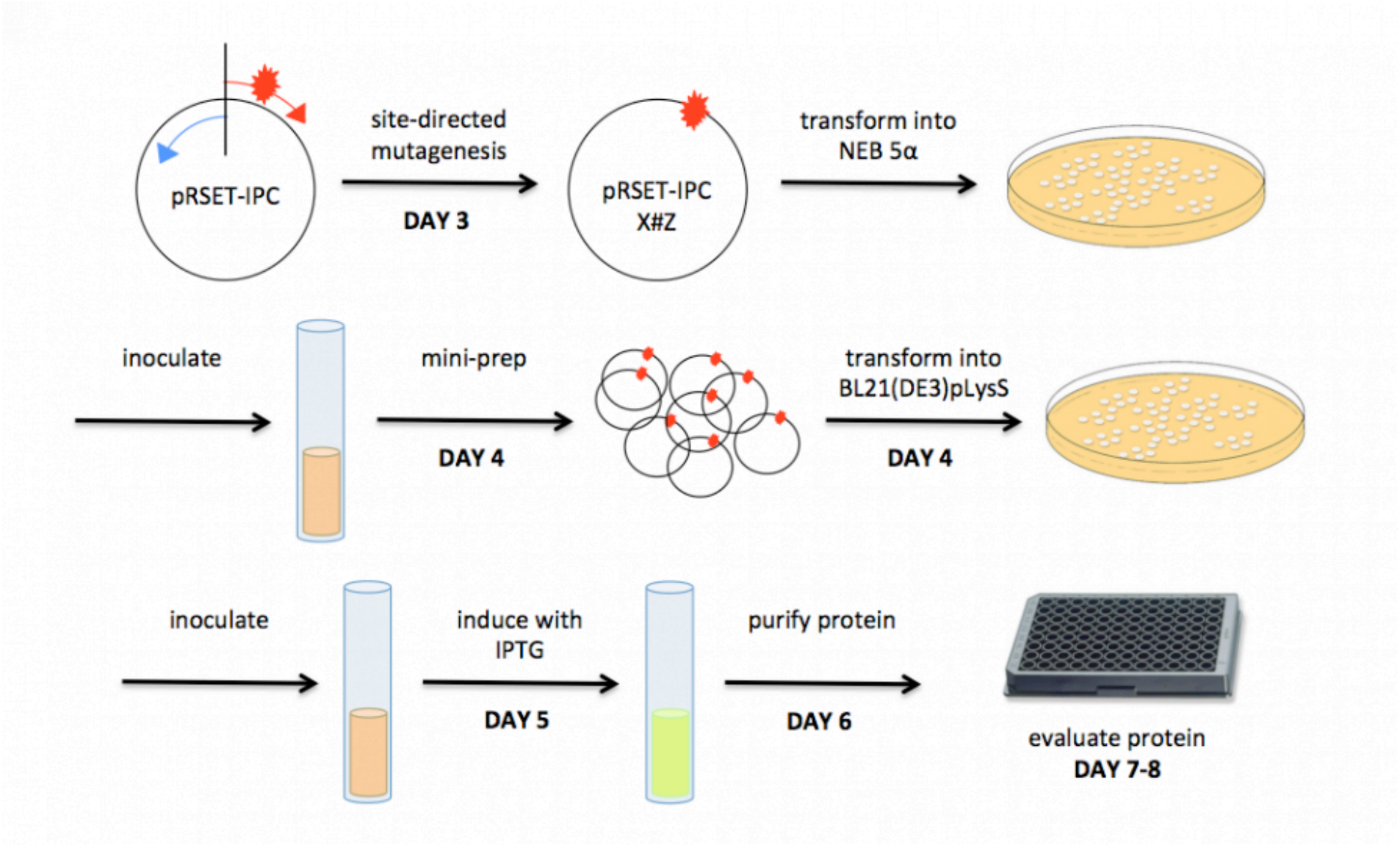
- Homework due M1D8:
  - Second round of methods (incorporating edits)
  - Optional mini-presentation outline
- on M1D8:
  - Quiz
  - Excel and Matlab analysis
  - Announce day Jing will grade notebook

MOD1 is coming to an end…

- Assessments for M1:
  - Protein engineering summary
    due at **5pm on Saturday, March 12th**
    revision due **5pm on Monday, March 28th**
  - Protein engineering presentation
    due at **10pm on Tuesday, March 15th**
  - blog post due 03/29 (discuss next time)

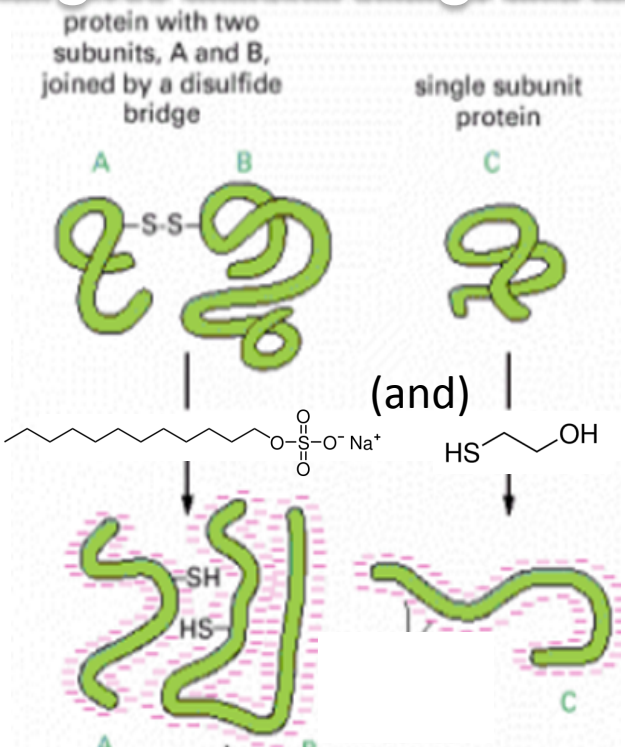
- **extra office hours in 56-302:**
  - All instructors Sunday 03/06, 10am-4pm
  - Noreen W 03/09 and R 03/10, 6pm-9pm
  - Maxine T 03/10 and F 03/11, 9am-11am
  - Leslie W 03/09 and F 03/11 2pm-5pm
- feedback on 03/17 from Noreen & Diana
- revision due 03/28

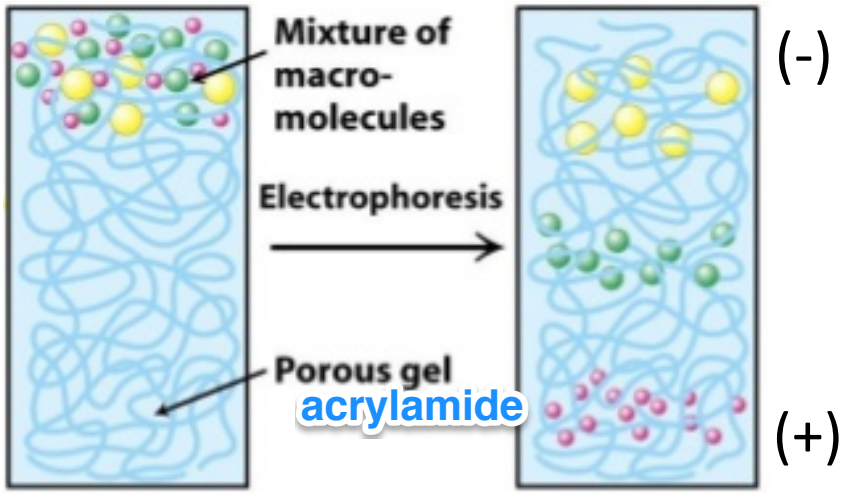
Last day of experiments for Module 1!



Sodium Dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

what gives uniform charge and linear structure?





BME

SDS

boiling

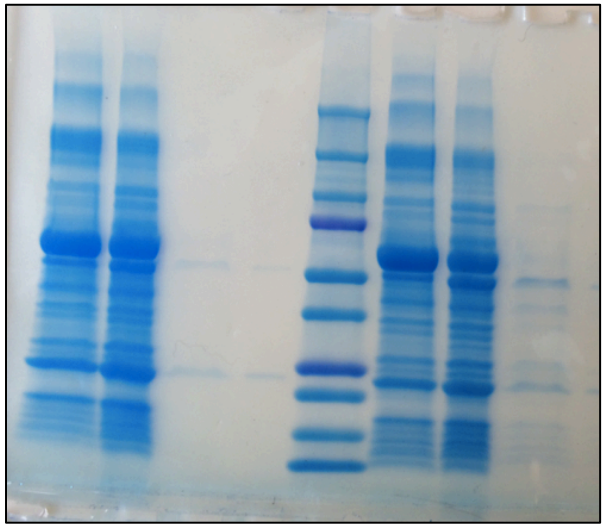
Proteins separated based on size

TGS:
Tris-HCl
Glycine
SDS

Cl-/protein/Glycine

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



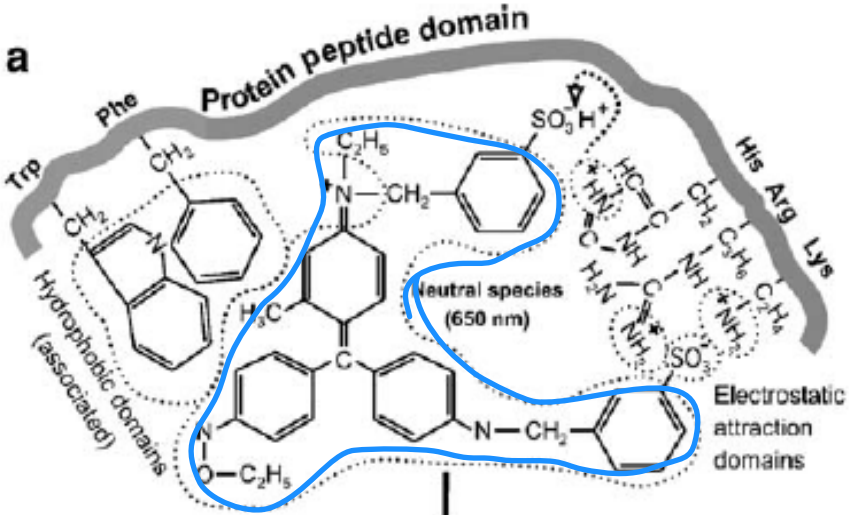
- 4-15% acrylamide gel:
  - for 10-250 kDa proteins
  - inverse pericam:

    bp = 1281 a.a. = 427 kDa 47

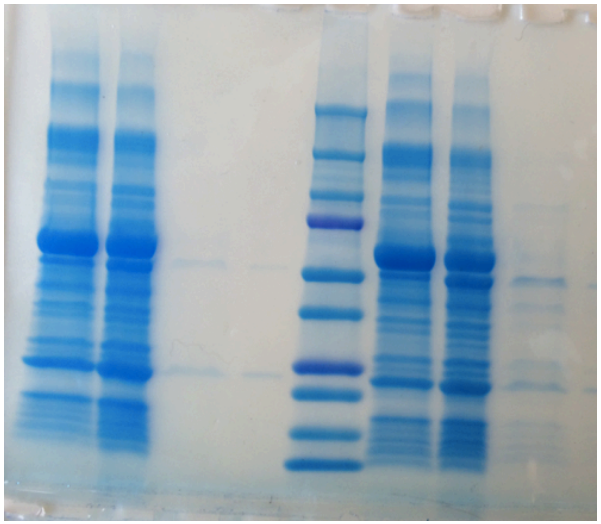
    ~ 110 Da / a.a
  - His-tag ~ 3 kDa 50kDa

Visualize proteins using Coomassie colorimetric assay

- Coomassie brilliant blue G-250 dye
  - red if unbound to protein (cationic form)
  - blue if bound to protein (anionic)
  - Van der Waals & hydrophobic interactions
  - Arg residues (also His, Lys, Phe, Trp)
  - absorption maximum 595nm (bright blue)

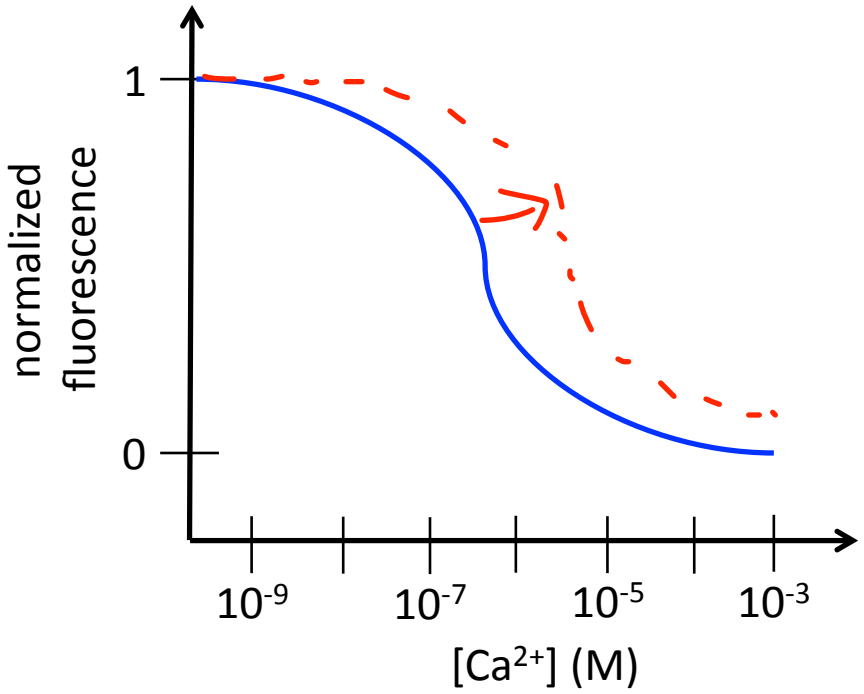


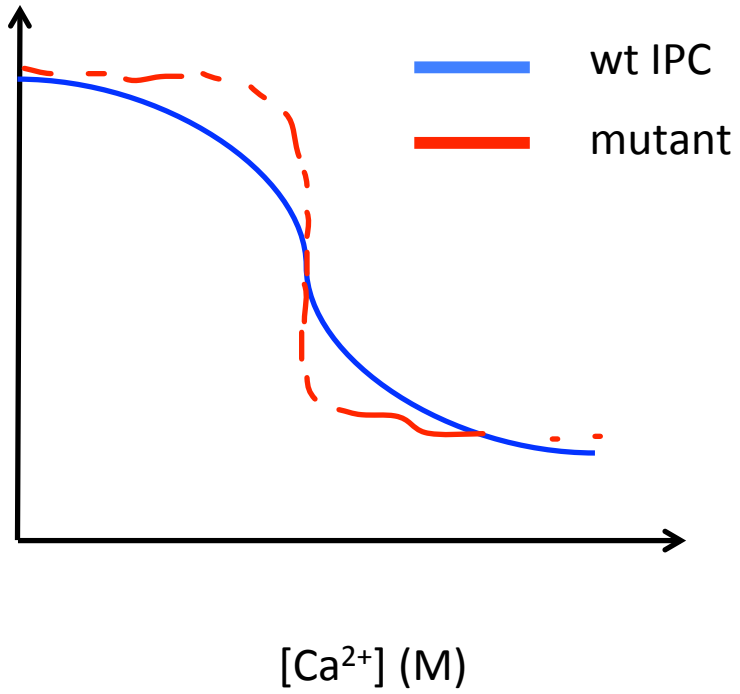
Coomassie



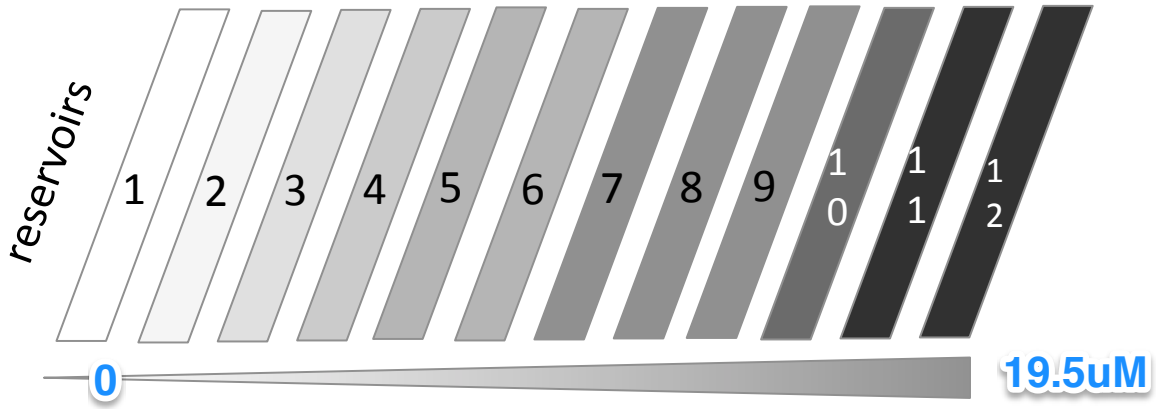
Protein engineering: Did your mutation affect IPC binding affinity and/or cooperativity

- **(only) affinity changes** — lower
- **(only) cooperativity changes** — higher



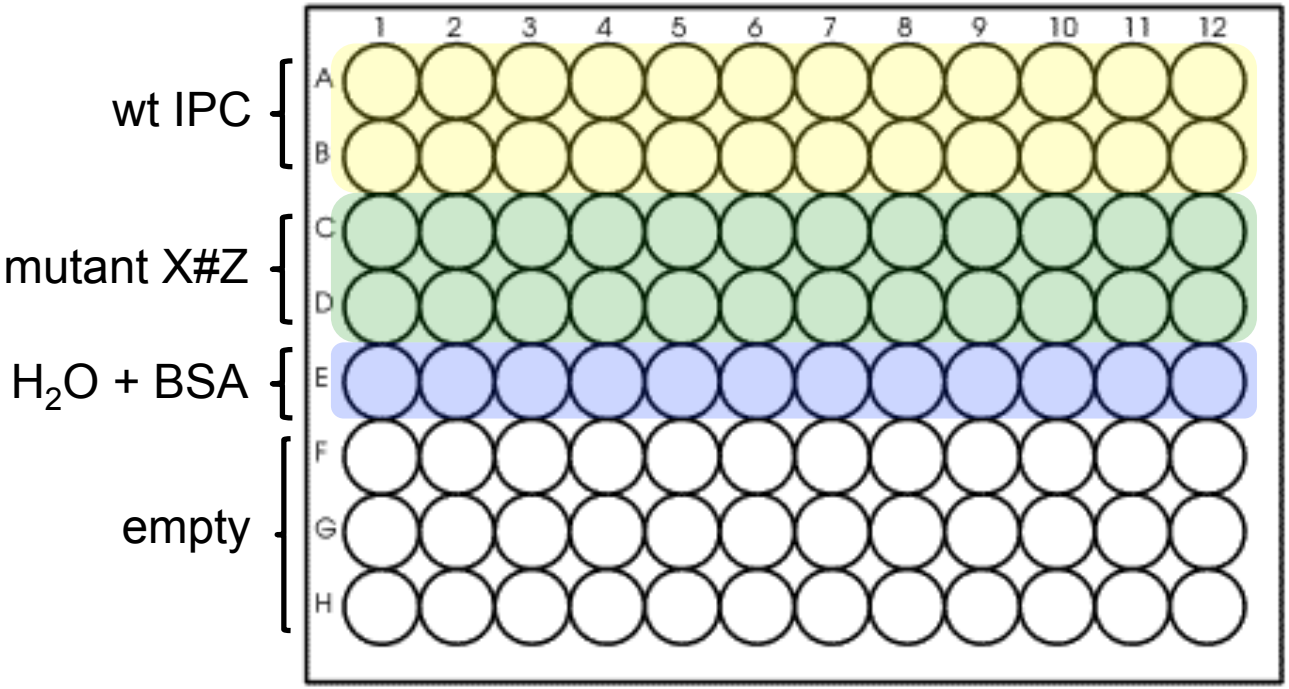


Black 96 well plate: reduces cross talk between wells

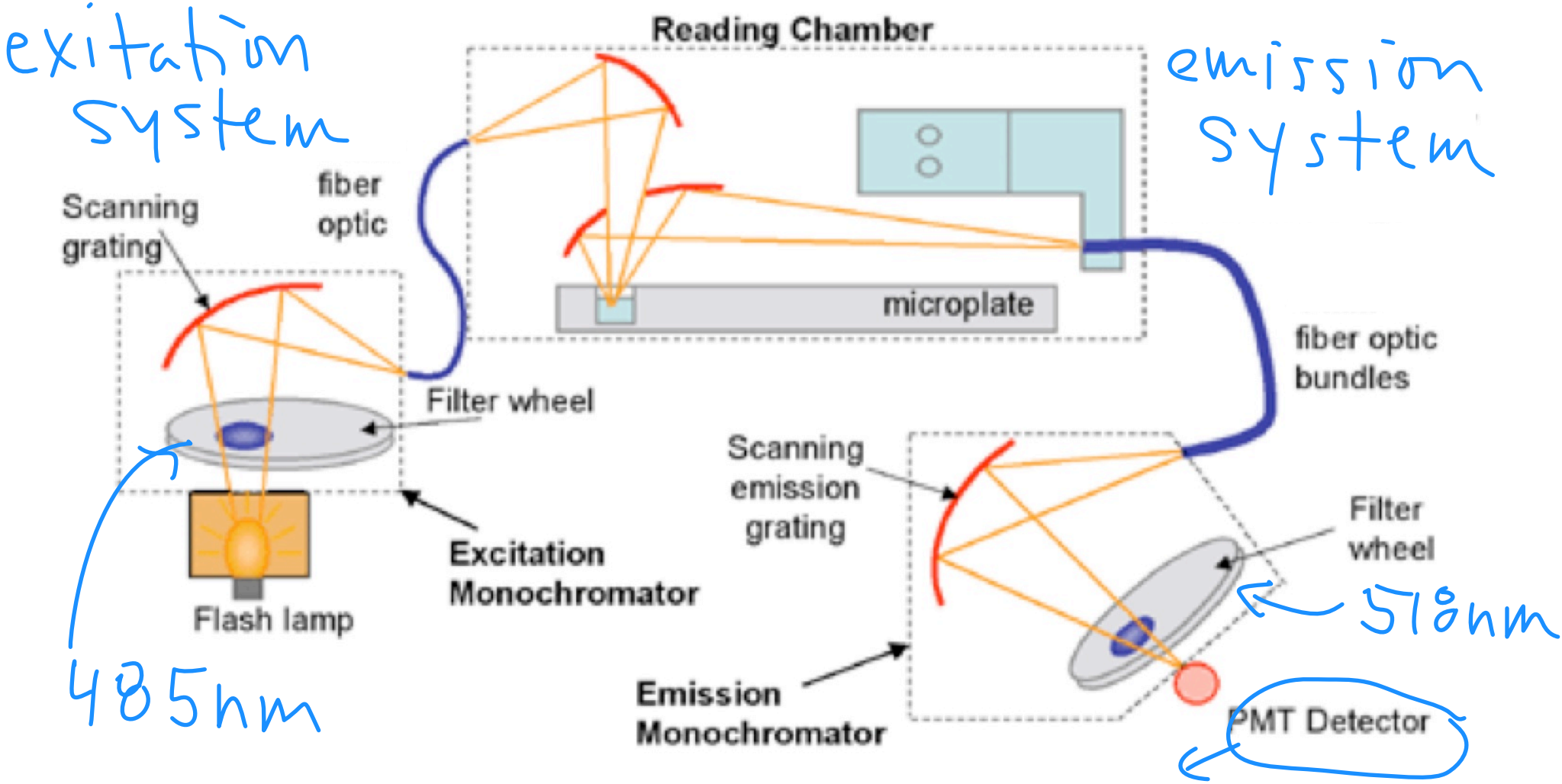


30ul pure protein (+BSA)

30l Ca2+ buffer mix



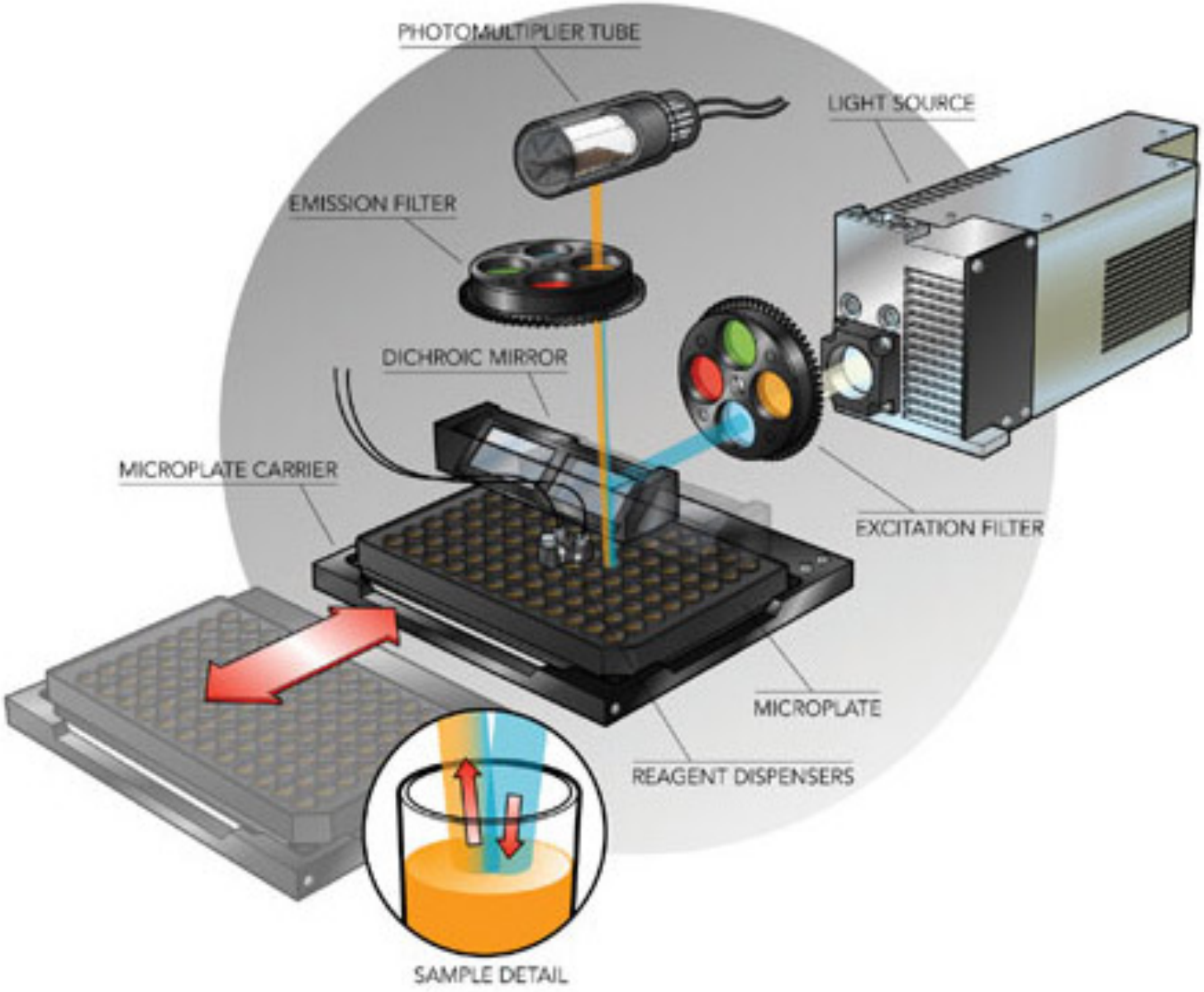
Fluorescence plate reader



adapted from keck.med.yale.edu

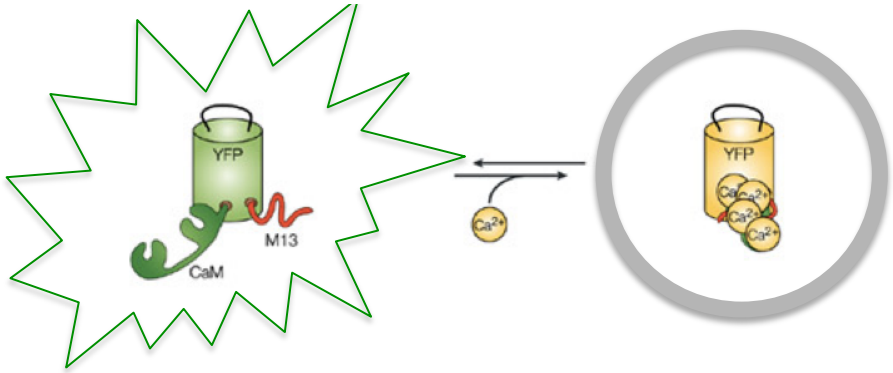
photomultiplier, measures signal using a light detector measures intensity!

Multimode Microplate Reader



Paul Held, Ph.D., BioTek Instruments.

Assay inverse pericam



- Varioskan Flash Spectral Scanning Multimode Reader
- 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 M1D8…
  - Excel
  - Matlab

Today in lab…

- Boil your SDS-PAGE samples prepared M1D6, spin down
- Load your samples on the gel, labeling your gel with a sticker, and run at 200V for 30-45min
- Prepare your calcium titration assay on a 96 well plate
- Rinse gel with water and stain with coomassie
- Take 96 well plate to the fluoresce plate reader for analysis
- Image coomassie stained gel on gel doc station