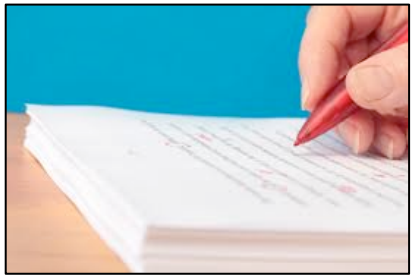
M1D7:
Characterize protein

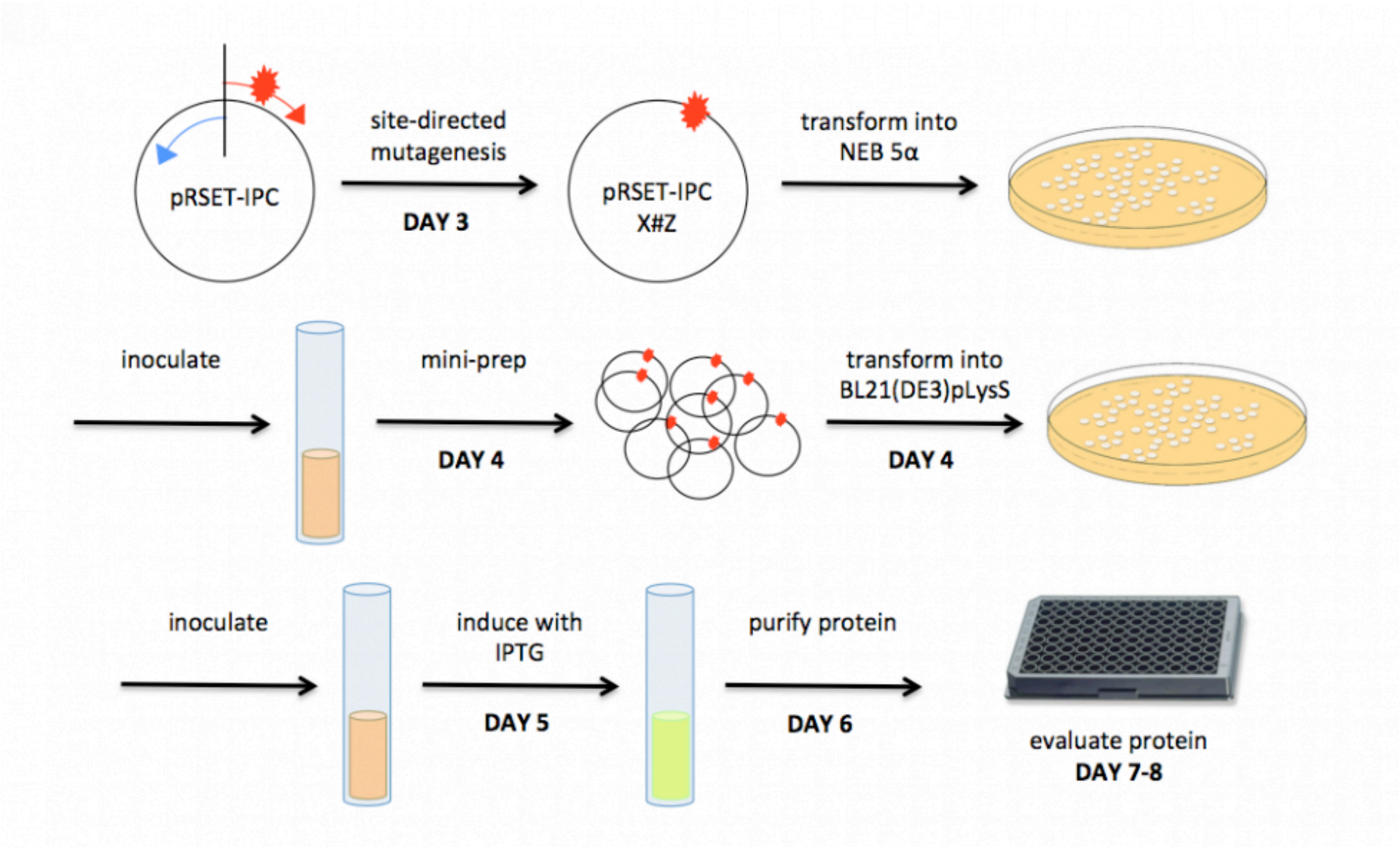
03/02/2016

Last week of M1!



- for M1D8:
  - revise and add to Methods section
  - *optional:* outline of mini-presentation
- on M1D8:
  - quiz
  - Jing (jgzhang@mit.edu) will grade lab notebook
- wrapping up M1:
  - protein engineering summary draft due 03/12
    - **extra office hours in 56-302:**
      All instructors Sunday 03/06, 10am-4pm
      Noreen W 03/09 and R 03/10, 6pm-9pm
      Leslie W 03/09 and F 03/11, 2pm-5pm
      Maxine T 03/10 and F 03/11, 9am-11am
    - feedback on 03/17 from Noreen & Diana
    - revision due 03/28
  - mini-presentation due 03/16
  - blog post due 03/29

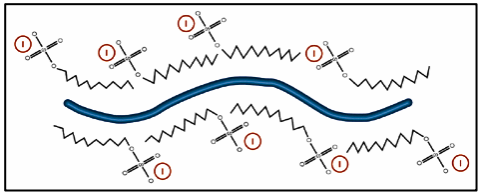
Last day of experiments for Module 1!

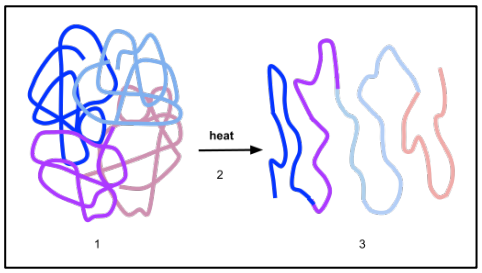


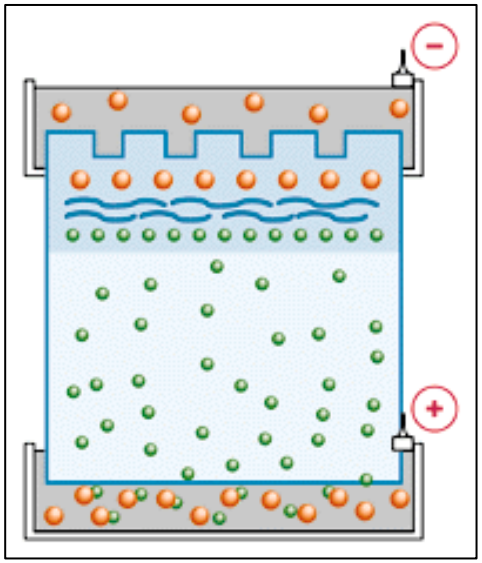
SDS-PAGE separates proteins by size

sodium dodecyl sulfate – polyacrylamide gel electrophoresis

carcinogenic

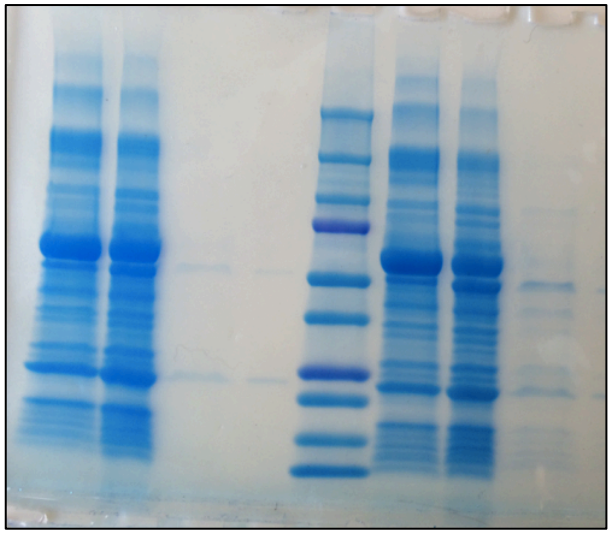






- Laemmli sample buffer / loading dye:
  - + SDS: detergent denatures proteins, coats proteins with negative charges
  - + β-mercaptoethanol reduces disulfide bonds
  - + bromophenol blue to follow front of migration
  - + glycerol
- boiling denatures higher-order structures
- TGS buffer : sandwiched proteins form tight bands
  - + Tris-HCl
  - + SDS
  - + 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

    stained helps follow migration

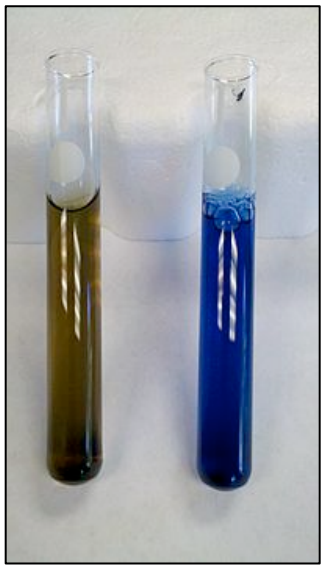
    unstained used for gross estimation of [IPC]
- 4-15% acrylamide gel:
  - for 10-250 kDa proteins
  - inverse pericam:

    1281 bp = **427** a.a. = **47** kDa

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

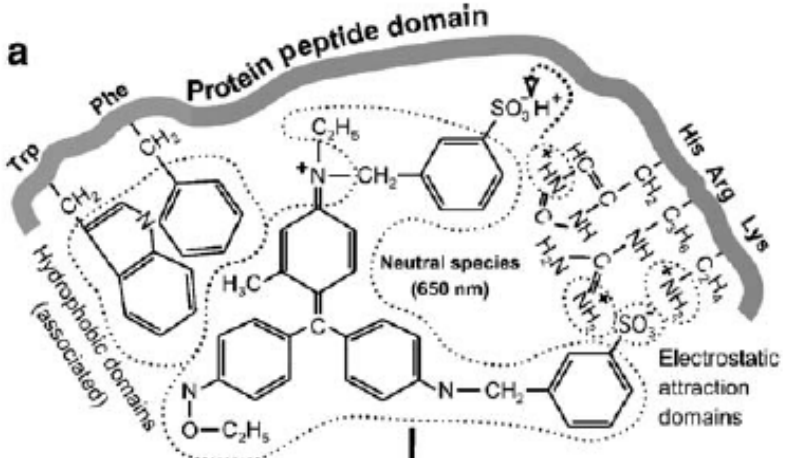
    IPC expected to appear as a 50 kDa band (47+3)

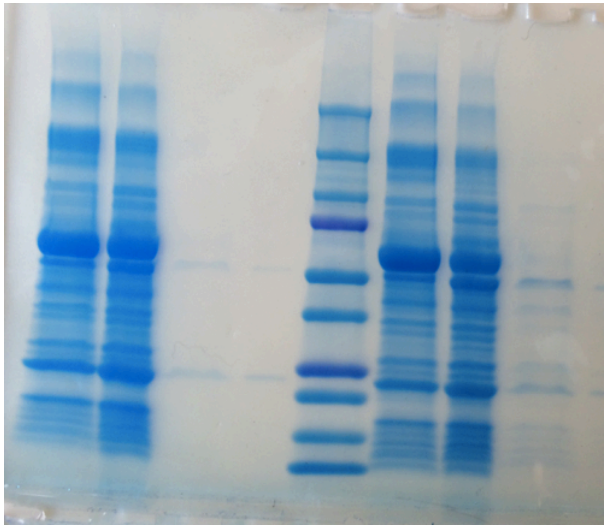
    BSA is 66 kDa



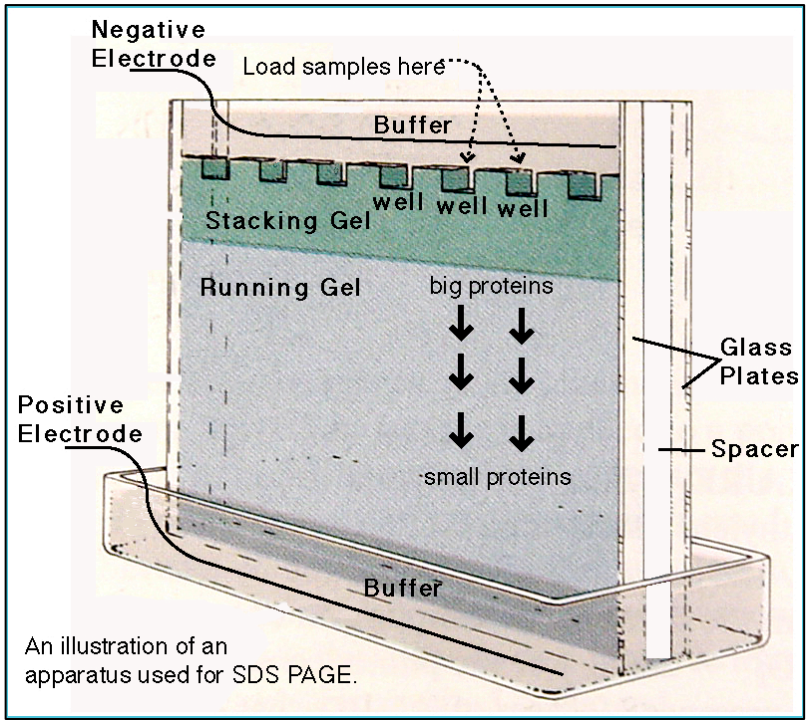
Visualize proteins using Coomassie colorimetric assay

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



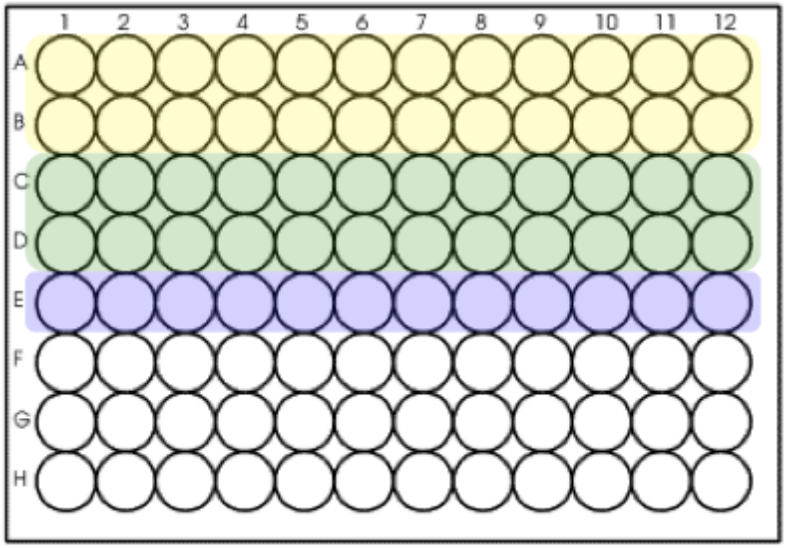


Georgiou *et al.* (2008) *Anal Bioanal Chem* **391**: 391-403



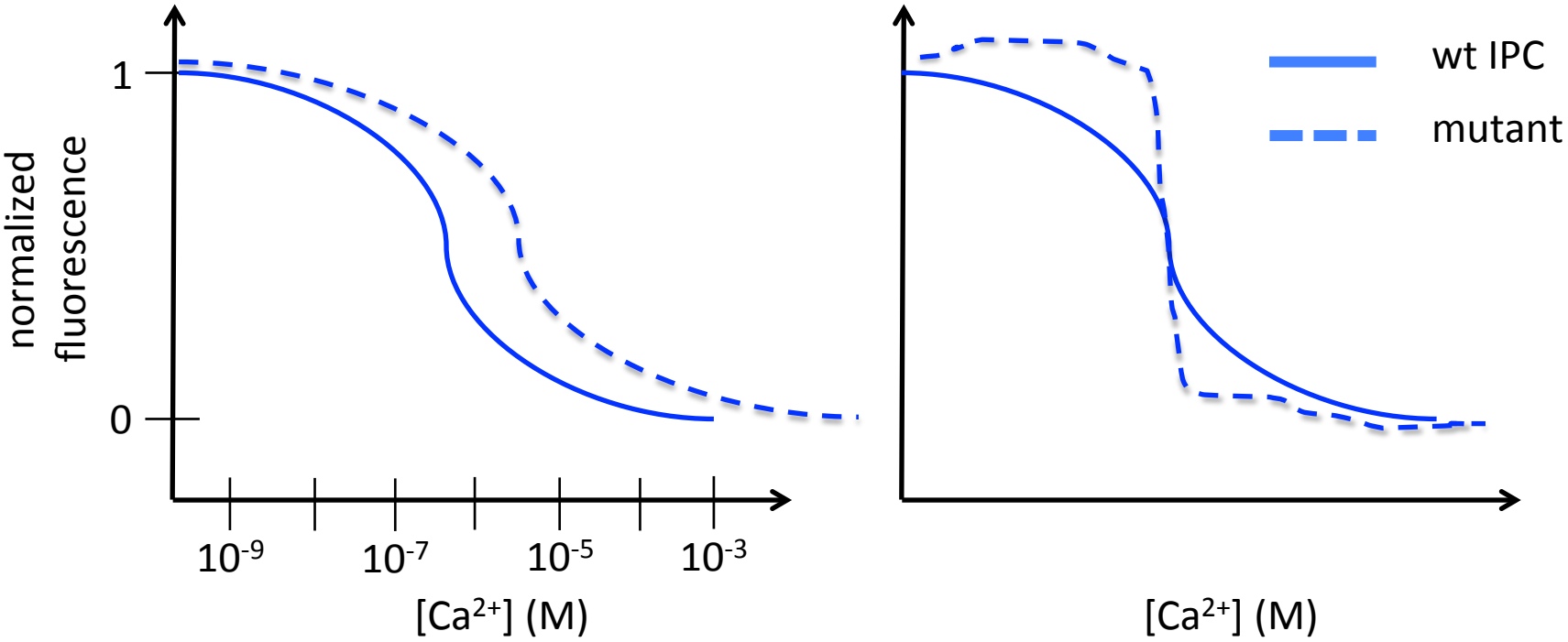
- SDS-PAGE
  - boil samples
  - load in lanes 2-9
  - run at 200 V for 30 min
  - rinse with water
  - stain with Coomassie

Today in lab



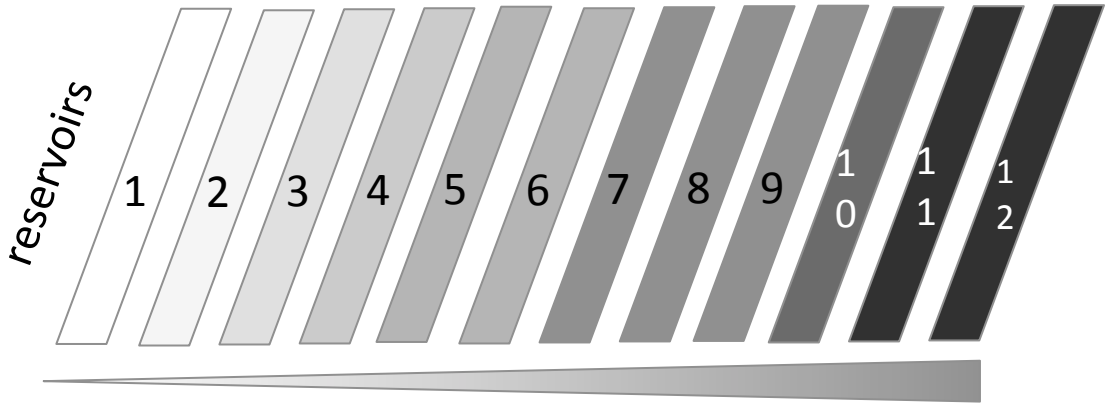
- IPC-calcium titration
  - prepare 96-well plate
  - read fluorescence levels with plate reader

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



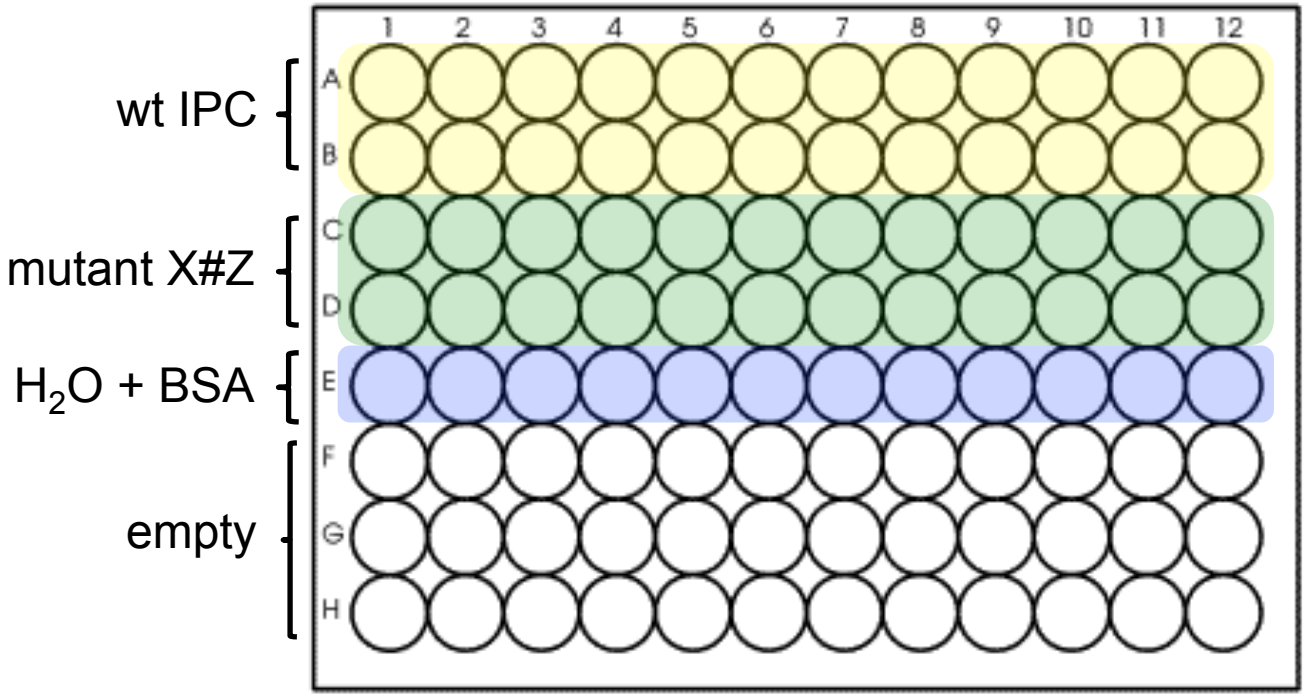
- (only) affinity changes
- (only) cooperativity changes

known concentrations of Ca2+
from 1 nM to 19.5 uM

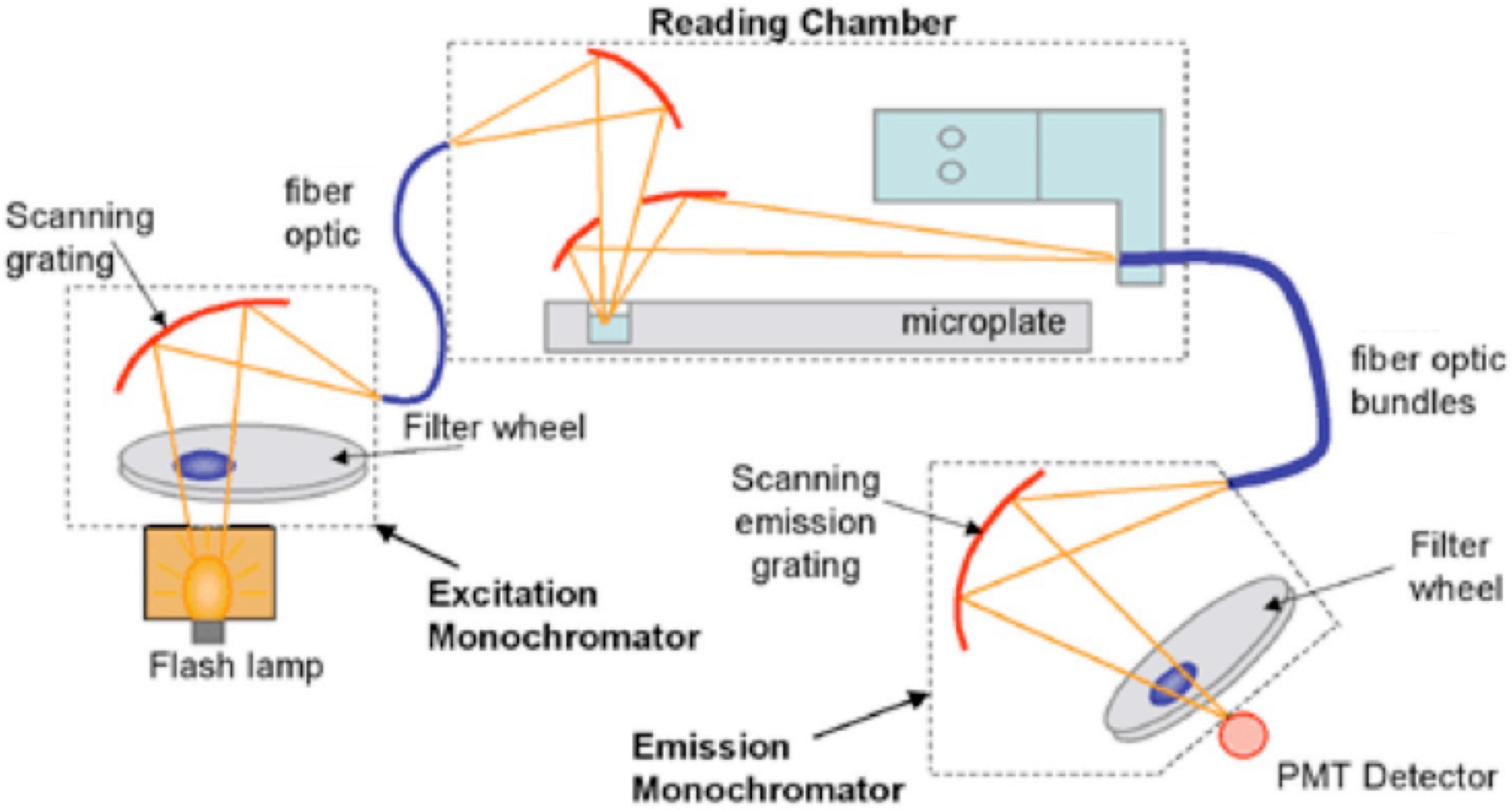


low
[Ca^{2+}]

high
[Ca^{2+}]

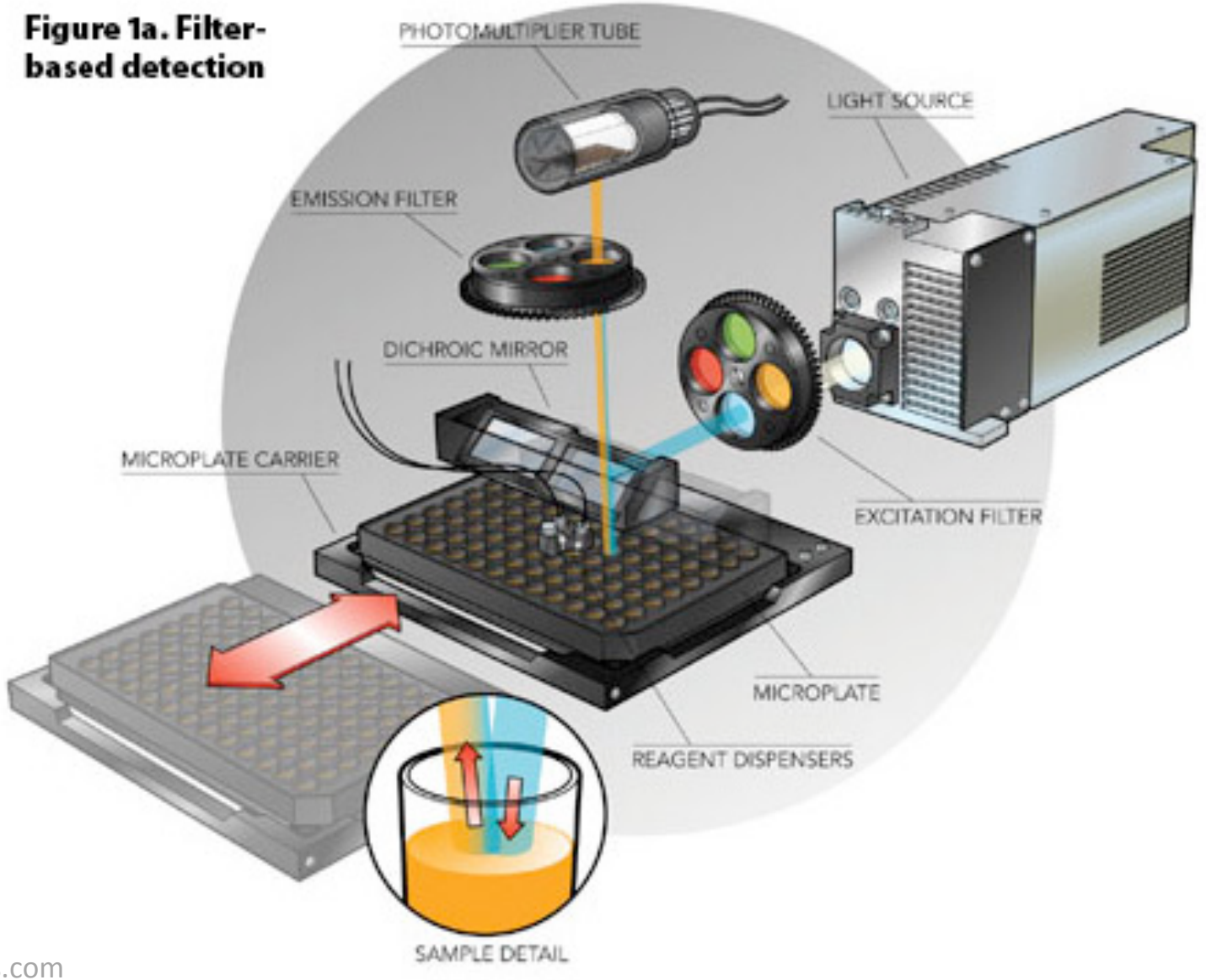


Fluorescence plate reader



adapted from keck.med.yale.edu

Microplate reader



genengnews.com

Assay inverse pericam

- Excitation: 485 nm
- Emission: 518 nm

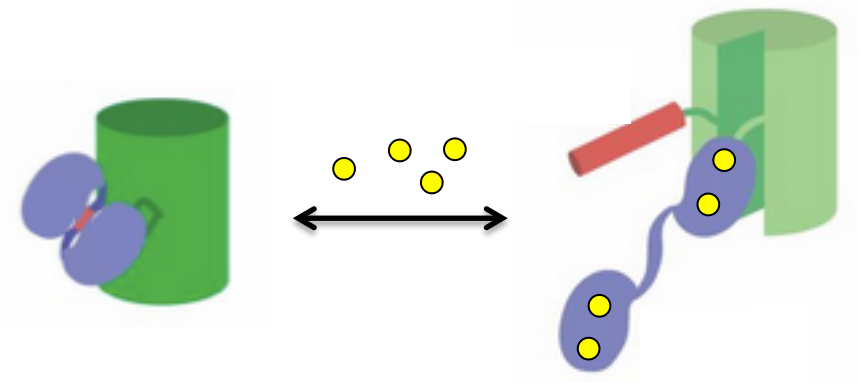
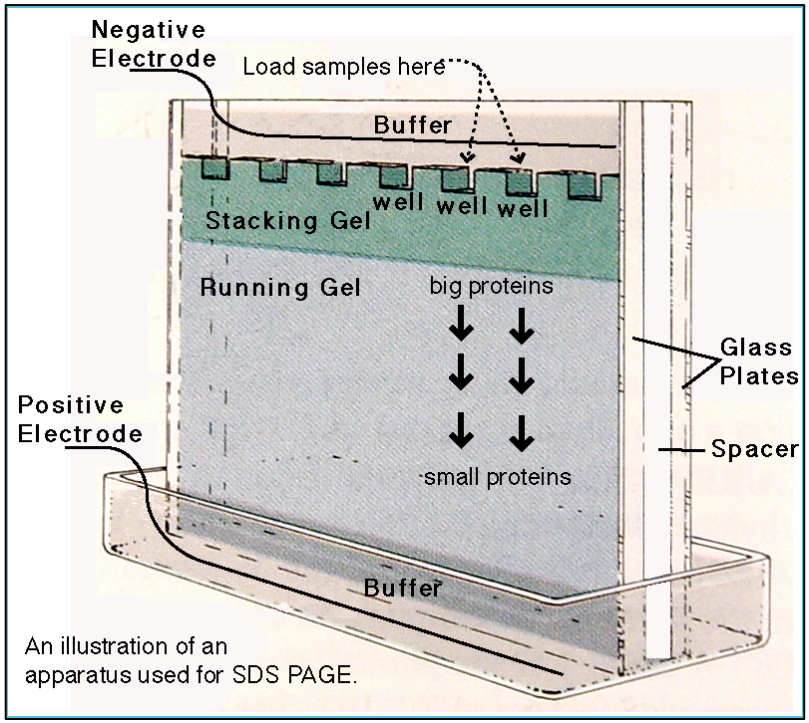


plate												
D132H	0.926	0.960	0.985	0.965	1.038	0.780	0.987	1.028	0.923	0.323	0.286	0.256
D132H	0.706	0.851	0.799	0.780	0.919	0.804	1.037	0.914	0.852	0.344	0.310	0.308
wt IPC	0.528	0.443	0.430	0.398	0.359	0.331	0.316	0.263	0.239	0.166	0.175	0.178
wt IPC	0.489	0.477	0.477	0.424	0.373	0.313	0.305	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 M1D8…
  - Excel
  - Matlab



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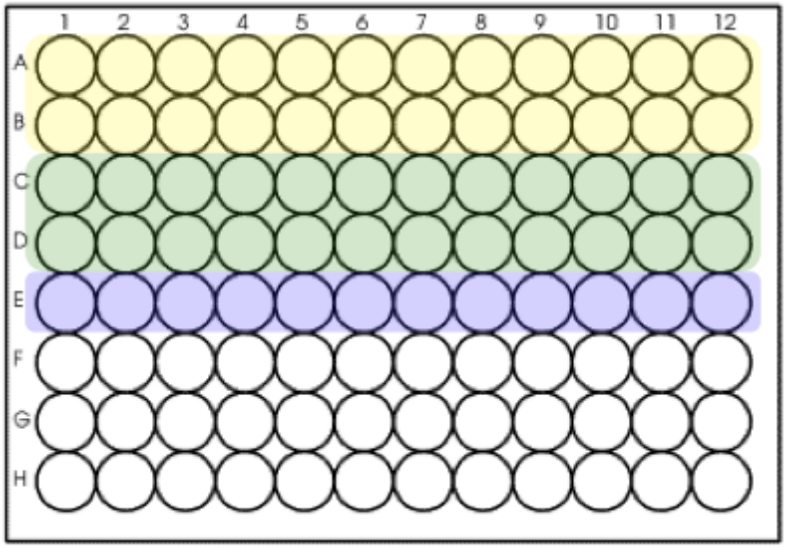

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