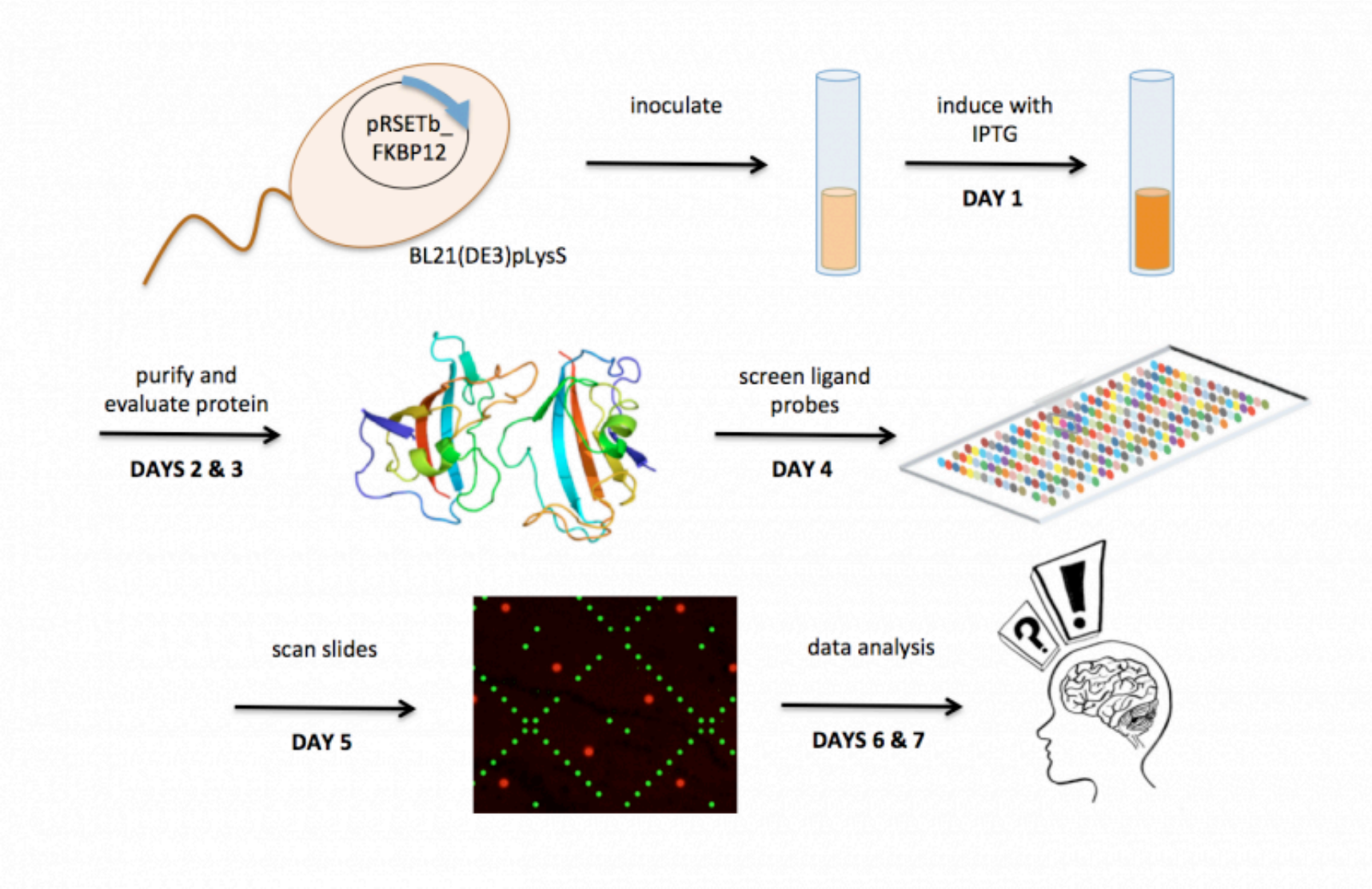
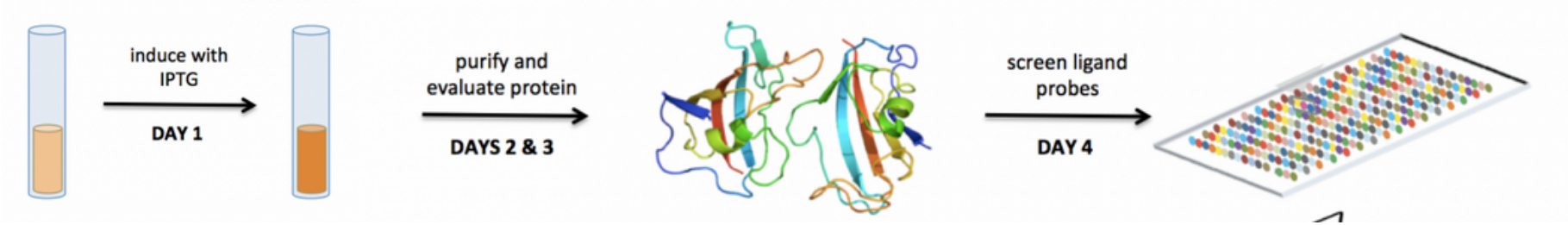
M1D3:
Evaluate purified protein

02/16/2017

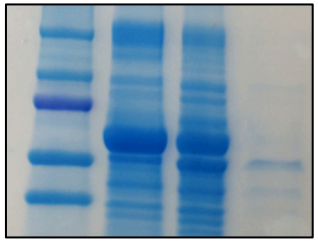
Overview of
“M1: High-throughput ligand screening”

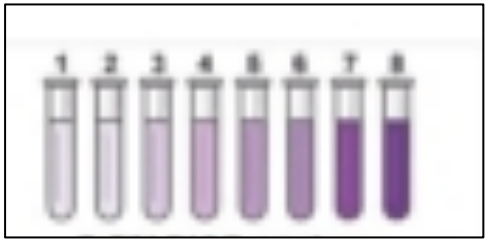


Let's also measure protein concentration



1. **SDS-PAGE**
- [FKBP12]
- protein purity
- leaky expression of FKBP12 under T7 promoter

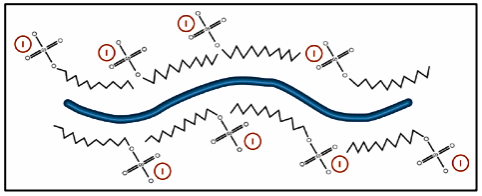


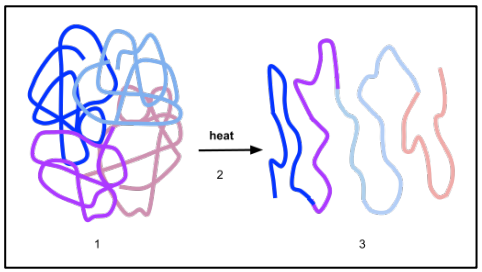


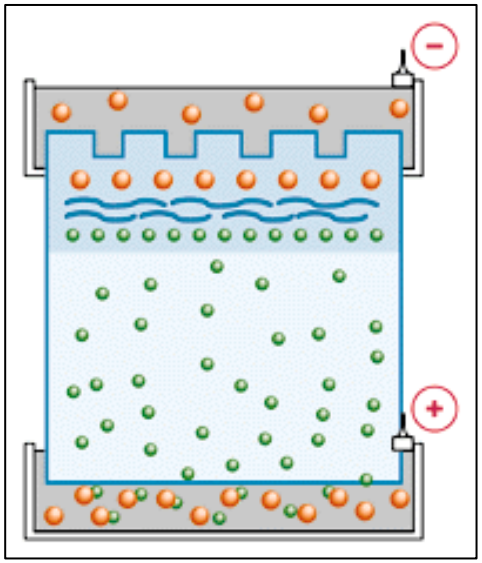
2. **BCA assay**
- [FKBP12]

1. SDS-PAGE separates proteins by size

sodium dodecyl sulfate – polyacrylamide gel electrophoresis







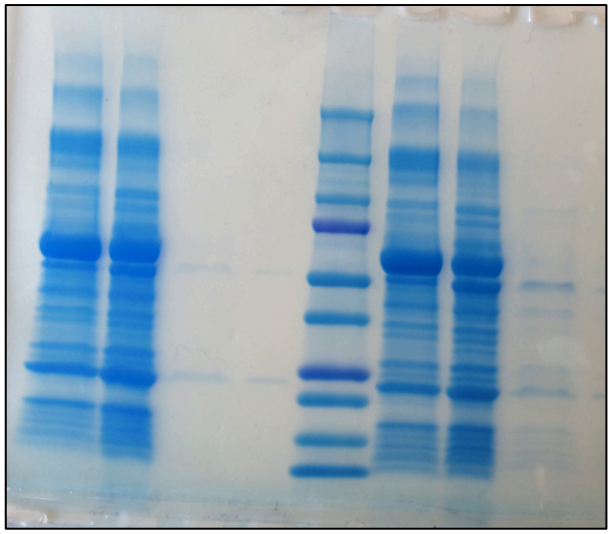
large

small

- Laemmli sample buffer / loading dye:
  - + SDS: detergent, denatures proteins; coats proteins with negative charge
  - + β-mercaptoethanol reduces disulfide bonds
  - + bromophenol blue
  - + glycerol
- boiling denatures higher-order structures
- TGS buffer
  - + Tris-HCl
  - + SDS
  - + glycine

https://www.nationaldiagnostics.com/electrophoresis/article/multiphasic-buffer-systems

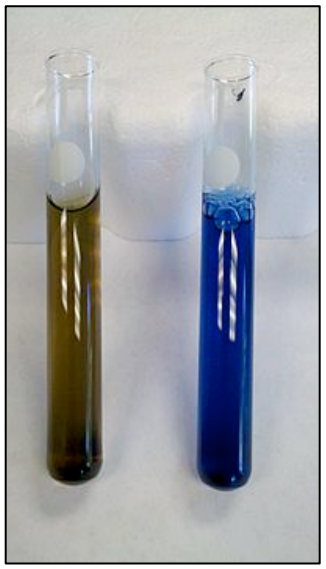
Load 6 samples + 2 ladders on SDS-PAGE gel



- Loading order:
  - think about figure(s) in your Results
  - cell lysate – IPTG / + IPTG
  - supernatant from 3 washes (+ IPTG)
  - purified, dialyzed FKBP12
  - stained and unstained ladders

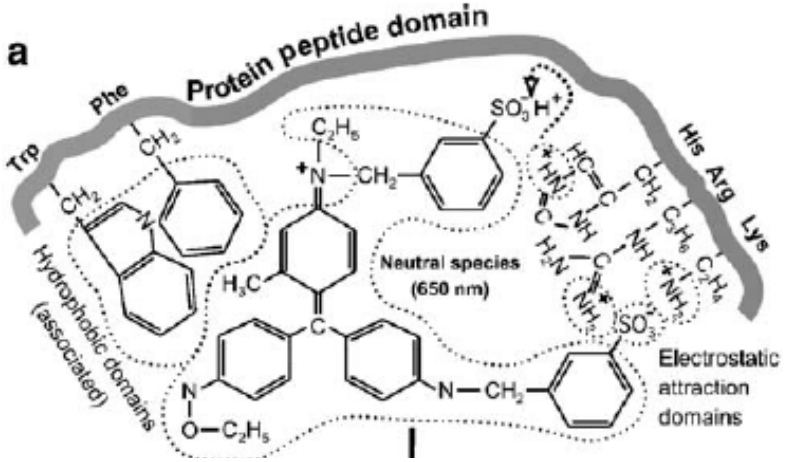
    stained: visual indicator of protein migration

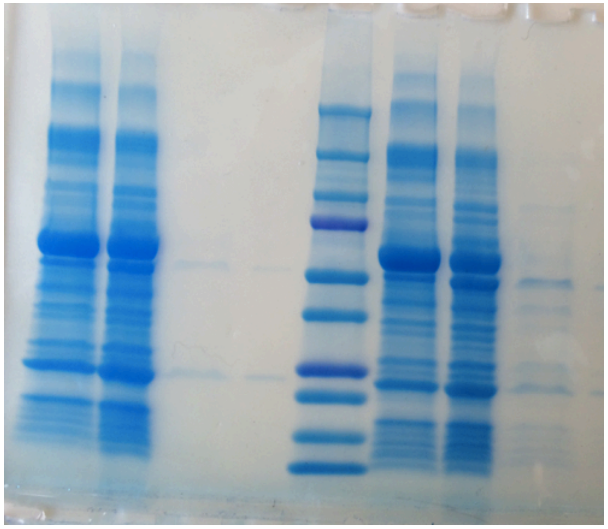
    unstained: known quantities of protein markers
- 4-15% acrylamide gel:
  - for 10-250 kDa proteins
  - FKBP12 ~ 12 kDa → 324 bp / 108 aa / ~ 110 Da / aa
  - His-tag ~ 3 kDa
  - expected band for 6His-FKBP12 ~ 15 kDa



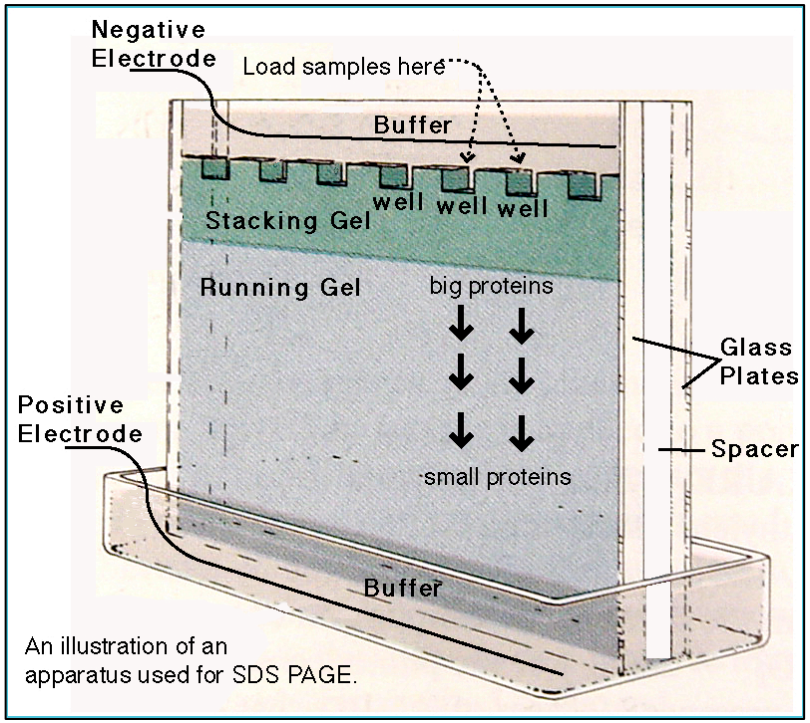
Visualize proteins using Coomassie colorimetric assay

- 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

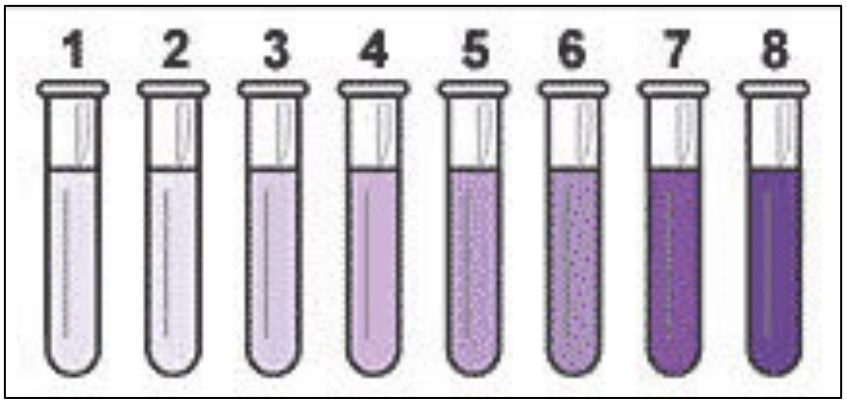




Georgiou *et al.* (2008) *Anal Bioanal Chem* **391**: 391-403



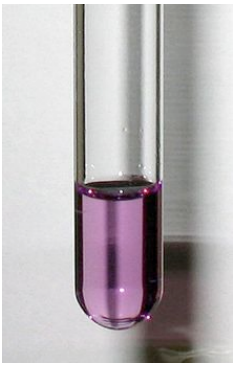
Today in lab



1. SDS-PAGE
   - boil samples
   - load in lanes 2-9
   - run at 200 V for 30 min 45 min
   - rinse with water
   - stain with Coomassie

2. BCA assay
   - prepare BSA standards
   - prepare working reagent
   - measure OD_{562} α protein concentration

The copper ion-based BCA (Smith) assay measures protein concentration



reduction

① from cupric (Cu^{2+}) to cuprous (Cu^{1+}) ions when binding to peptide (alkaline + temperature, Biuret reaction)
   - proportional to [protein]

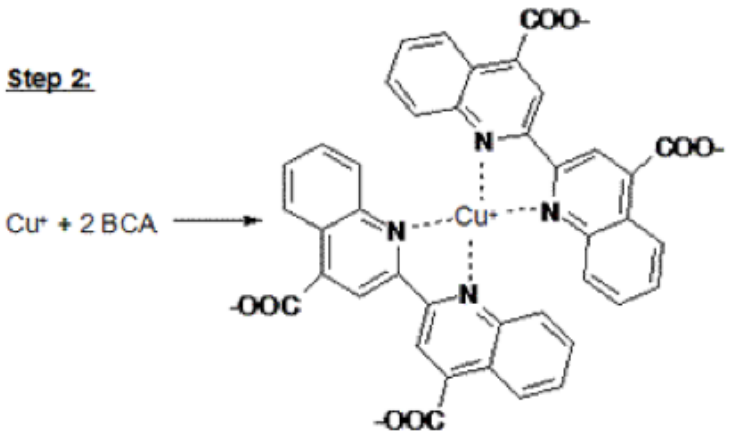
② Cu^{1+} reduces bicinchoninic acid (B**C**A)
   - BCA turns violet = absorbs 562 nm

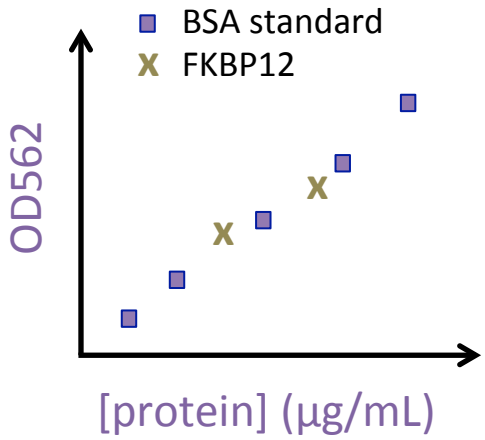
- calibration with bovine serum albumine (B**S**A)
  - 5 – 250 µg/mL

Step 1:

$$\text{Protein} + Cu^{2+} \xrightarrow{OH^-} \text{Protein} + Cu^{+}$$

Step 2:





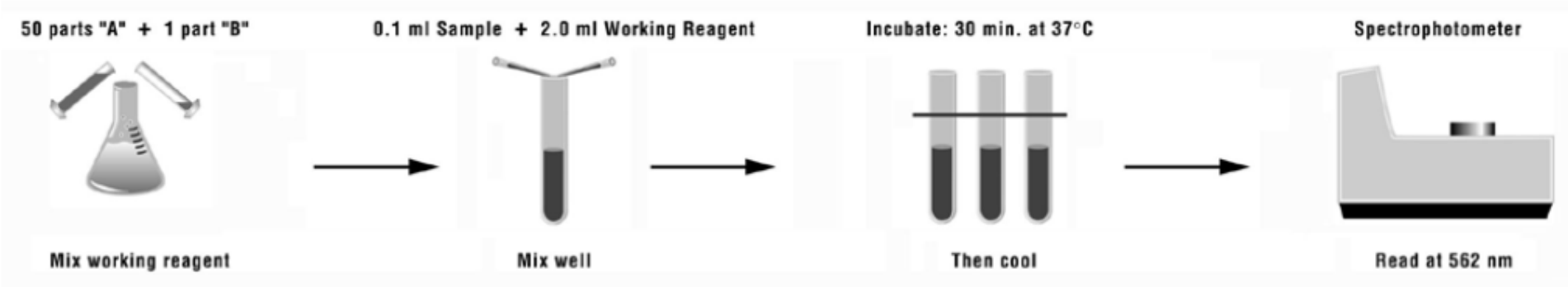
There exists several protein concentration assays

assay	absorption	mechanism	detection limit	advantages	disadvantages
UV absorption	280 nm	tyrosine and tryptophan absorption	0.1-100 ug/ml	small sample volume, rapid, low cost	incompatible with detergents and denaturating agents, high variability
Bicinchoninic acid	562 nm	copper reduction (Cu^{2+} to Cu^{1+}), BCA reaction with Cu^{1+}	20-2000 ug/ml	compatible with detergents and denaturating agents, low variability	low or no compatibility with reducing agents
Bradford or Coomassie brilliant blue	470 nm	complex formation between Coomassie brilliant blue dye and proteins	20-2000 ug/ml	compatible with reducing agents, rapid	incompatible with detergents
Lowry	750 nm	copper reduction by proteins, Folin-Ciocalteu reduction by the copper-protein complex	10-1000 ug/ml	high sensitivity and precision	incompatible with detergents and reducing agents, long procedure

Table 1. Common total protein assays.

Be careful!

- Fresh tips from dilution to dilution
- Mix well
- (C) differs from others ☺



M1D4 is after Presidents' Day!

- MIT BE
  BIOLOGICAL ENGINEERING
  COMMUNICATION LAB

- 1 figure = 1 message
  - either SDS-PAGE *or* BCA graph
  - remember title & caption
- Methods
  - M1D2
  - M1D3

find Comm workshop slides on wiki

Office hours

Noreen Lyell

- M 2-5
- in 16-317

in addition, next week:

T 3-5pm 02/21
W 3-5pm 02/22

Leslie McClain

- T 9:30-11
- in 56-341c

T 9:30am-12:30pm

Maxine Jonas

- R 9:30-11
- in 16-239

M 1-3pm 02/20