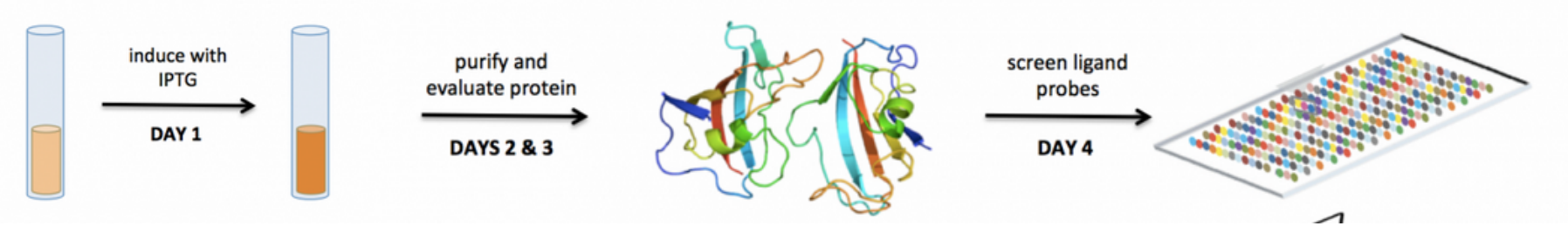
M1D3:Evaluate purity and concentration of FKBP12

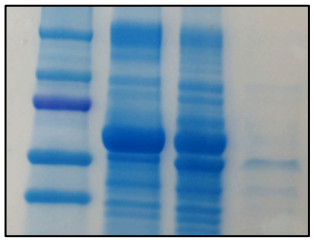
02/17/2017

1. Quiz
2. Communication Workshop
3. Prelab discussion
4. SDS-PAGE + Coomassie stain
5. BCA assay

Let's assess protein purity and concentration



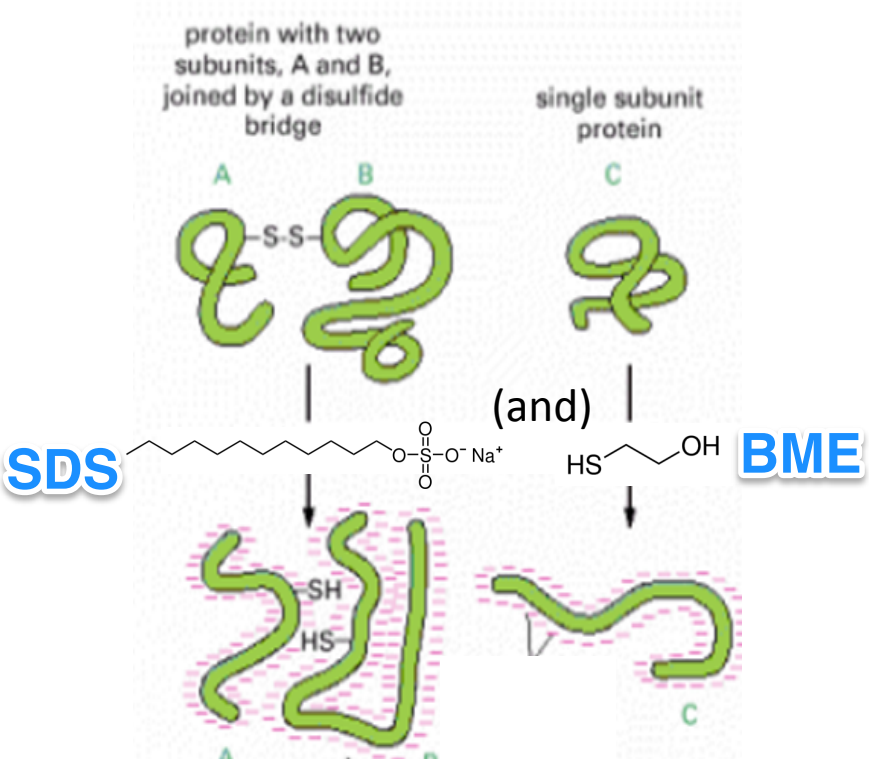
1. **SDS-PAGE**
   - [FKBP12]
   - protein purity
   - leaky expression of FKBP12 under T7 promoter



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

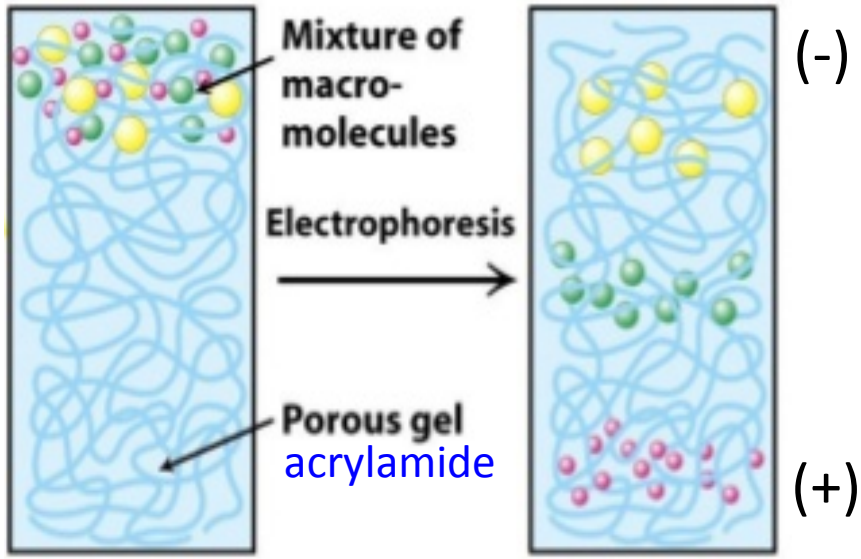
Sodium Dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

What gives proteins uniform charge and linear structure?



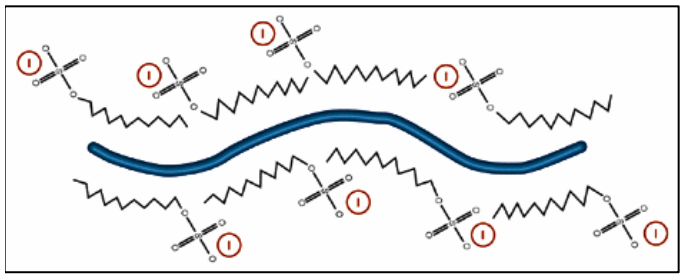
boiling

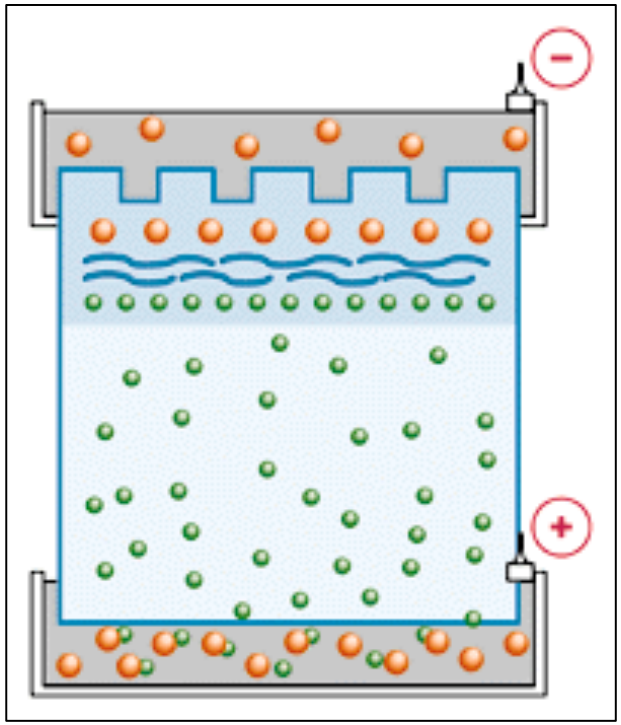
How are the proteins separated?



After mixed with Laemmli buffer all proteins are linear and negatively charged therefore proteins separated by size

SDS-PAGE separates proteins by size



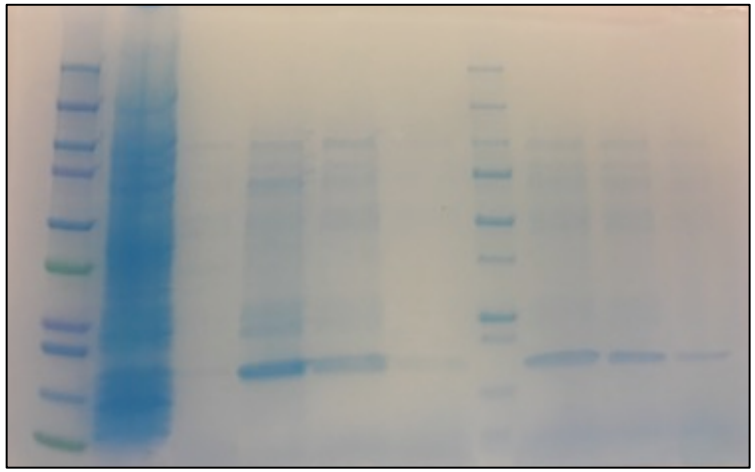


- Laemmli sample buffer / loading dye:
  - + SDS: **detergent, denatures proteins and coats with negative charge**
  - + β-mercaptoethanol **breaks disulfide bonds**
  - + bromophenol blue **dye that runs at 3-5kDa**
  - + glycerol **viscosity, helps proteins sink in well**
- boiling denatures higher-order structures
- TGS buffer **TGS buffer "sandwiches" proteins as they enter the gel**
  - + Tris-HCl
  - + SDS, protein
  - + glycine

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

Load 7 samples + 2 ladders on SDS-PAGE gel

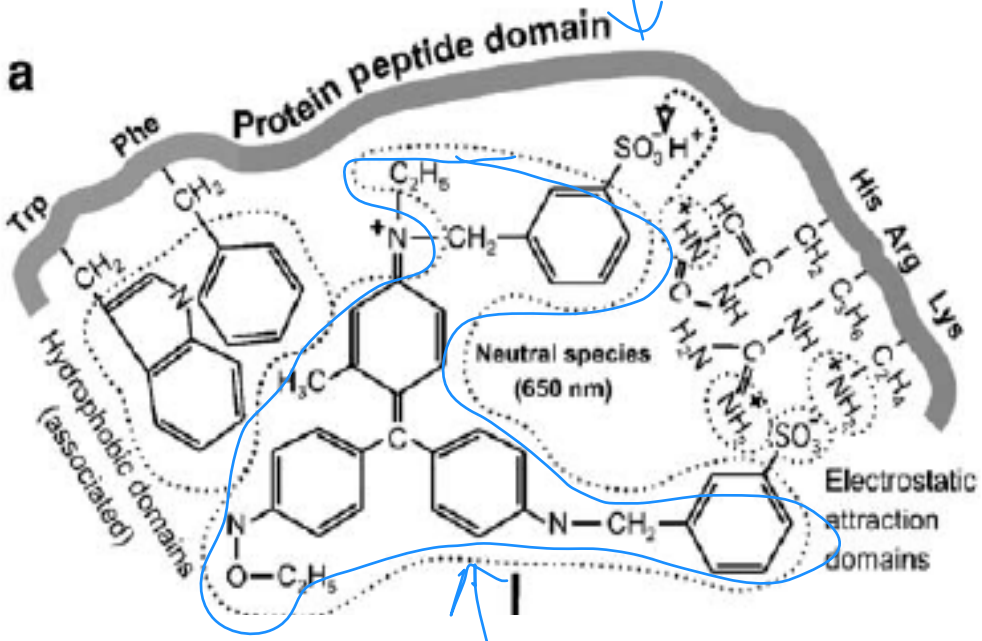
- Loading order:
  - **think about figure(s) in your Results**
  - cell lysate – IPTG / + IPTG
  - supernatant from 3 washes (+ IPTG)
  - elution before dialysis
  - dialyzed FKBP12
  - stained and unstained ladders



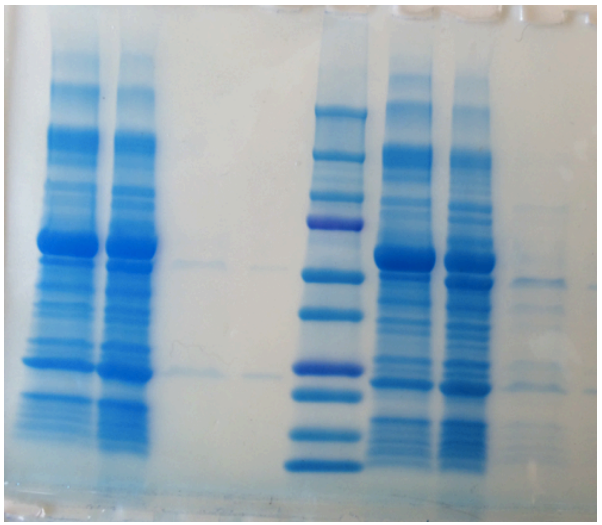
- 4-15% acrylamide gel:
  - for 10-250 kDa proteins
  - FKBP12 ~ 12 kDa
    - 324bps = 108aa= ~110 Daltons/aa
  - His-tag ~ 3 kDa
  - 6His-FKBP12~ 15kDa

Visualize proteins using Coomassie colorimetric assay

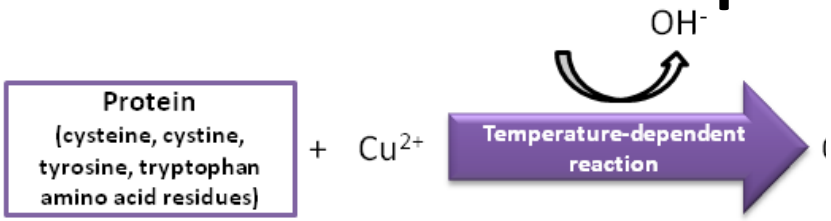
- Coomassie brilliant blue G-250 dye
  - red if unbound (cationic form)
  - blue if bound to protein (anionic)
  - Van der Waals & hydrophobic interactions
  - Arg residues (also His, Lys, Phe, Trp)

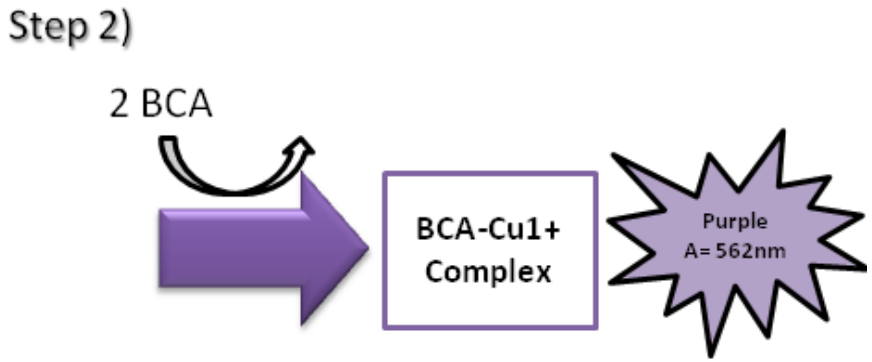


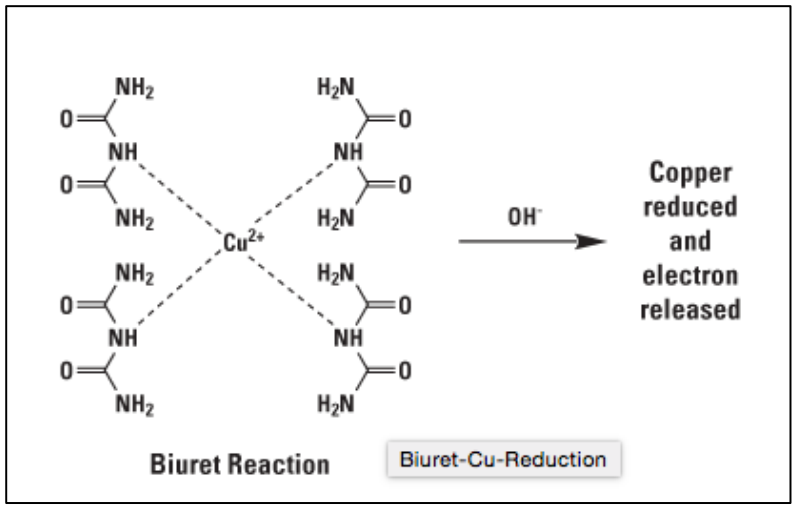
Georgiou *et al.* (2008) *Anal Bioanal Chem* **391**: 391-403



BCA protein assay

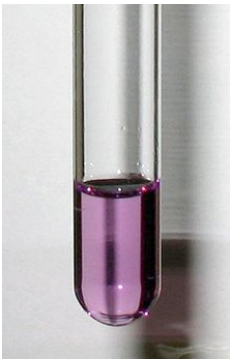






- BCA: bicinchoninic acid is a chromogenic reagent used to detect Cu^{1+}
- amount of Cu^{2+} reduced to Cu^{1+} is a function of protein concentration (also temperature dependent)
- BCA mixed with Cu^{1+} results in purple which absorbs at 562 nm

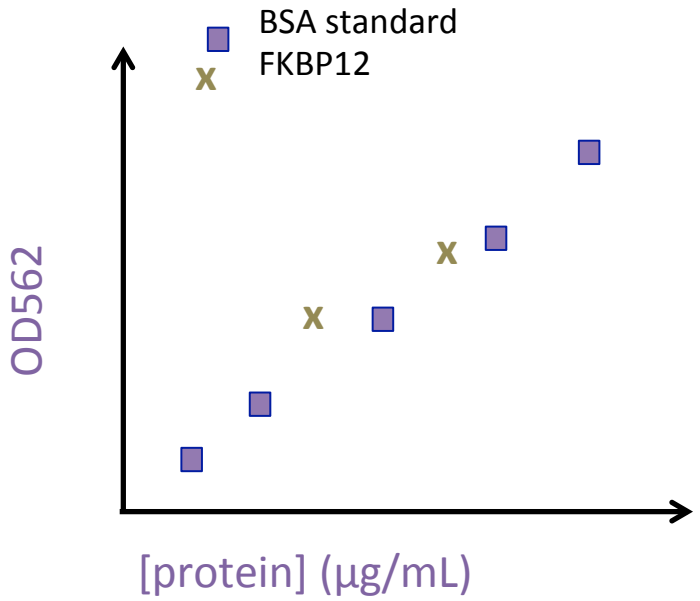
The copper ion-based BCA (Smith) assay measures protein concentration



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 (BCA)
   - BCA turns violet = absorbs 562 nm

- calibration with bovine serum albumine (BSA)
   - 5 – 250 µg/mL

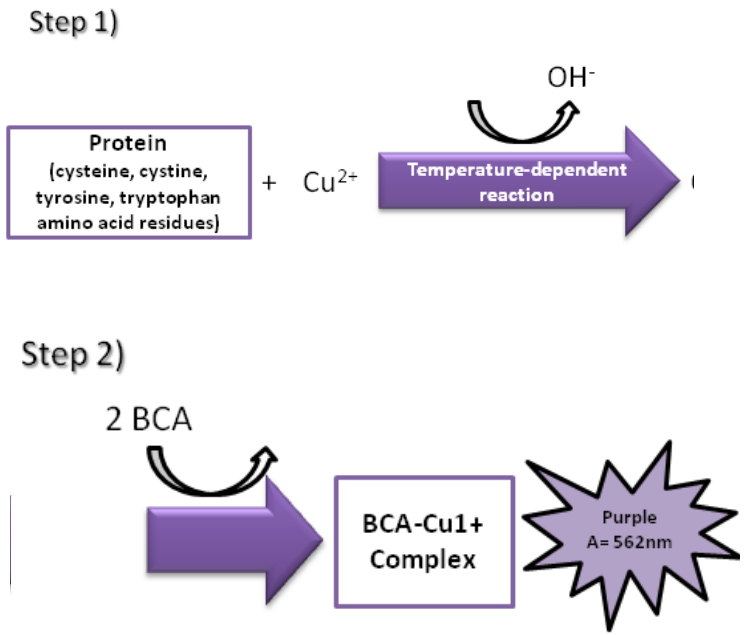


BCA protein assay

State of most of the copper reagent if….

1) No protein in your elution (Cu^{2+} / Cu^{1+})
2) 10ug/ml protein in your elution (Cu^{2+} / Cu^{1+})
3) You didn't heat your Copper/Protein mix (Cu^{2+} / Cu^{1+})
4) You had protein in your elution but didn't add BCA reagent before reading your sample on the spectrophotometer (Cu^{2+} / Cu^{1+})

but no color change!



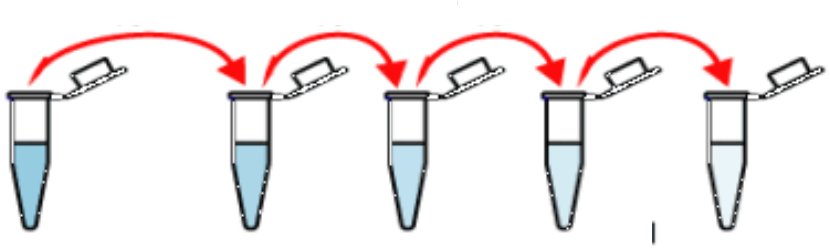
There exists several protein concentration assays

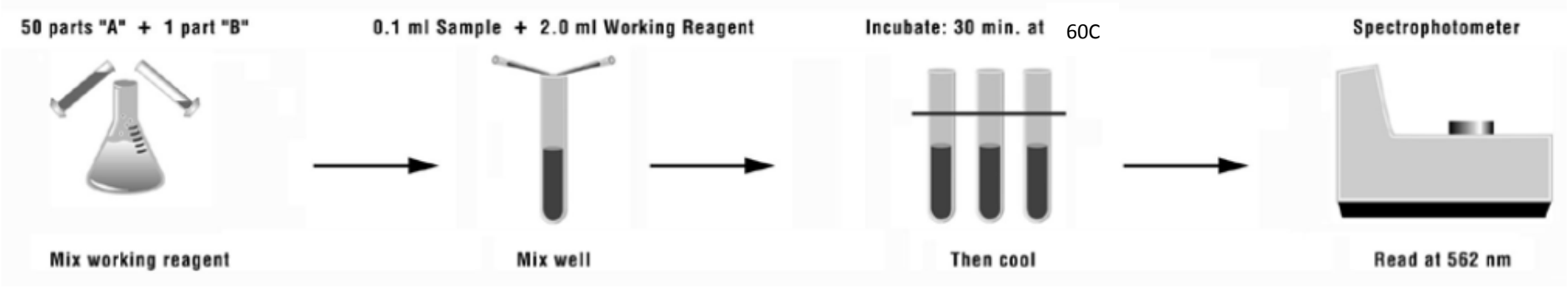
assay	absorption	mechanism	detection limit	advantages	disadvantages
UV absorption	280 nm	tyrosine and tryptophan absorption	0.1-100 ug/ml	small sample volume, rapid, low cost	incompatible with detergents and denaturating agents, high variability
Bicinchoninic acid	562 nm	copper reduction (Cu^{2+} to 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 between dilutions
- Pay careful attention to volumes





M1D4 is a week from today!
Start your homework ASAP…

- Data Figure, represents evaluation of purified protein
  - 1 figure = 1 message
  - either SDS-PAGE *or* BCA graph
  - remember title & caption
- Methods *(will send out more details via email)*
  - Induction M1D1 (Part 2 and Part 4 only)
  - M1D2
  - M1D3
- Meet with BE fellow, submit a short summary about your meeting(1-2 paragraphs)

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

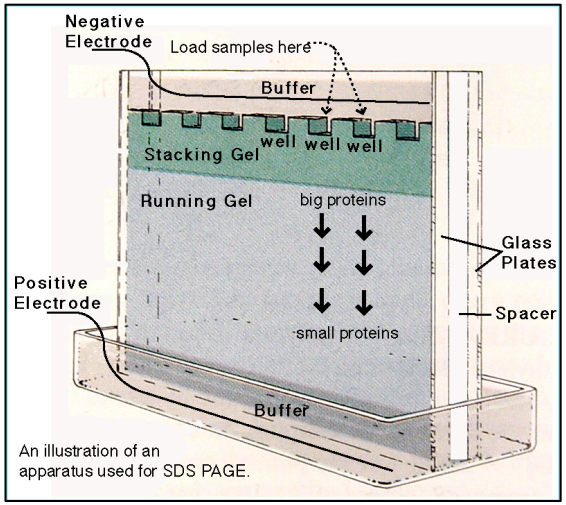
T 9:30am-12:30pm

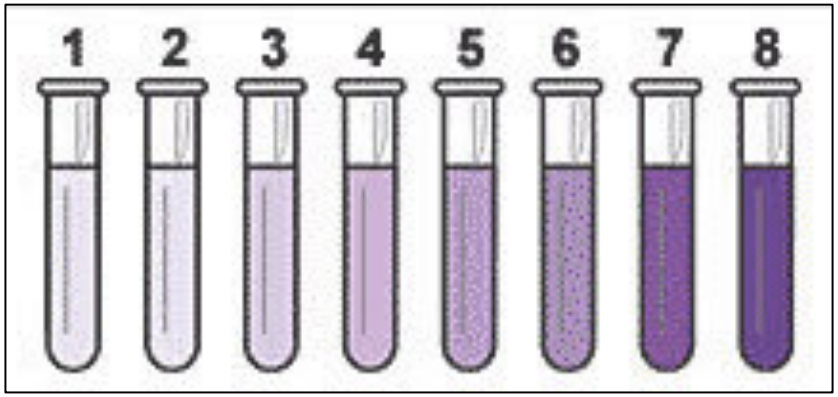
Maxine Jonas

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

M 1-3pm 02/20

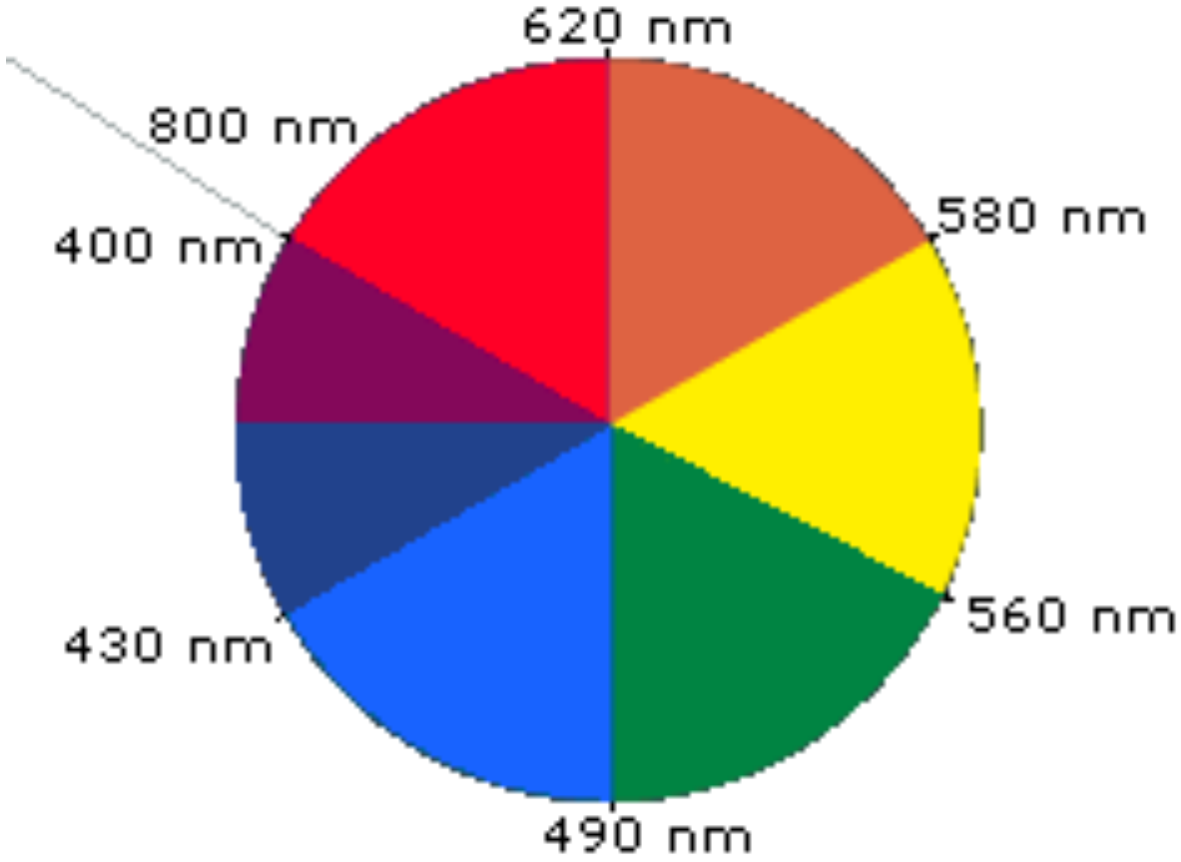
Today in lab:





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

2. BCA assay
   - prepare BSA standards
   - prepare working reagent
   - incubate at 60°C for 30min
   - measure OD_{562}



https://www2.chemistry.msu.edu, Visible and Ultraviolet Spectroscopy