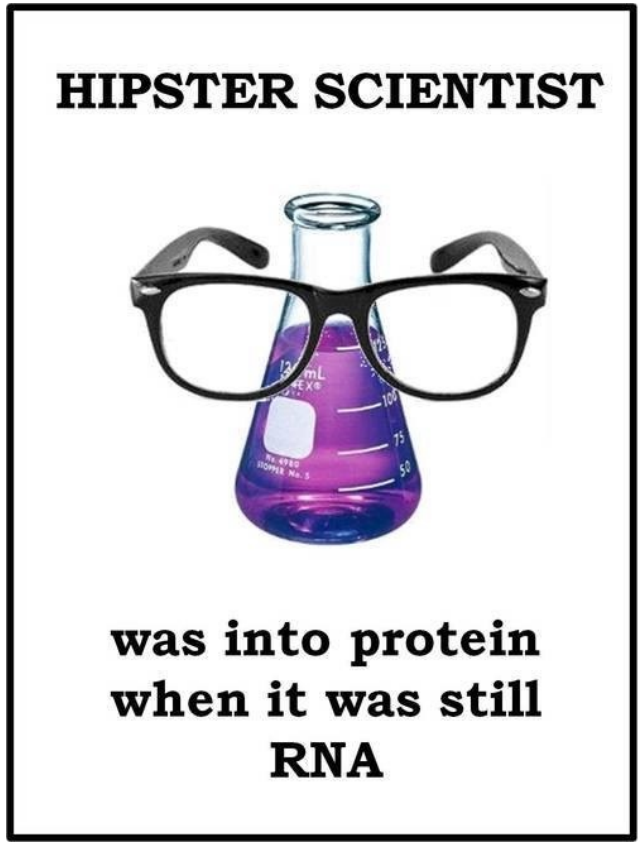
M1D3:
Assess purity and concentration of purified protein

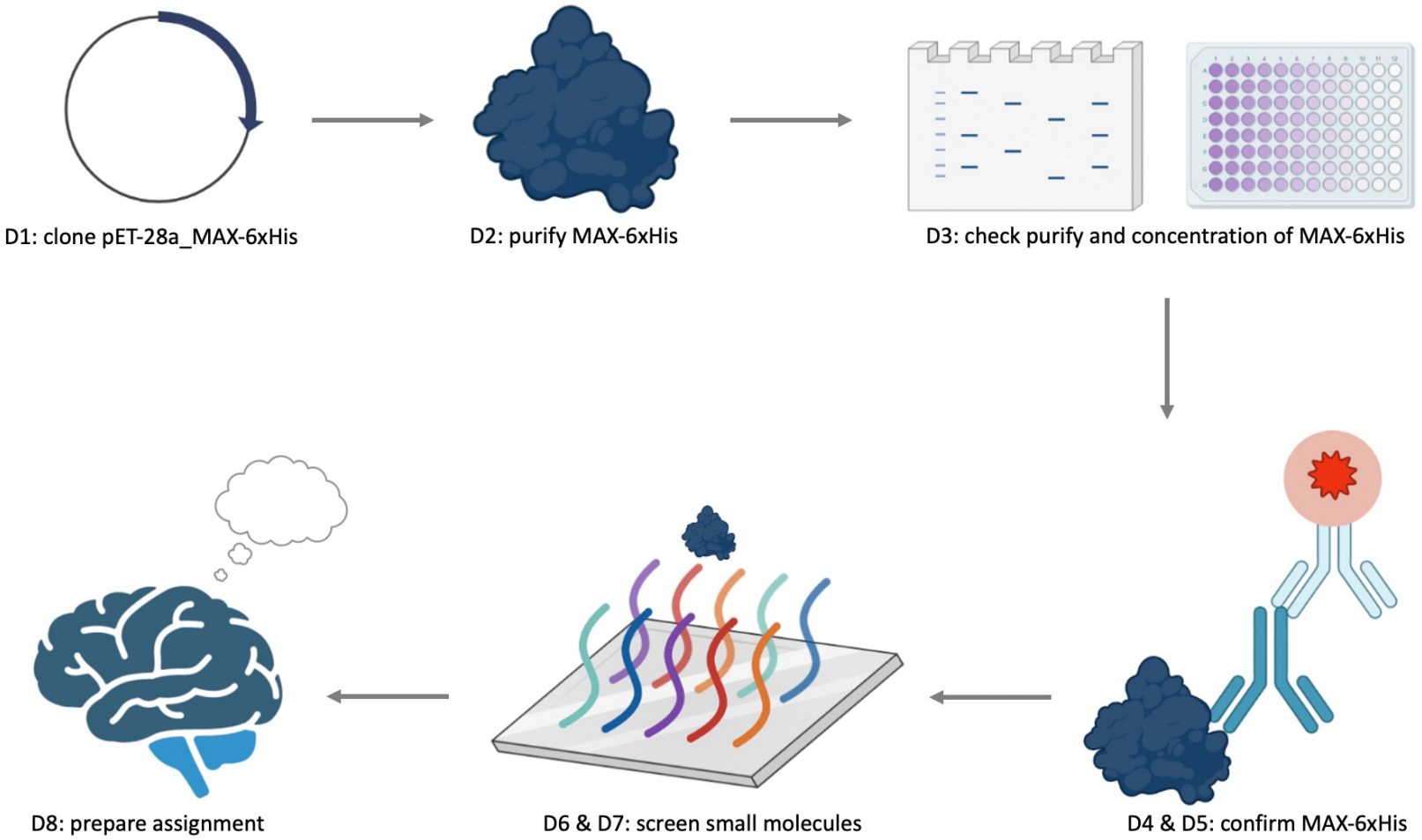
1. Comm Lab workshop
2. Prelab discussion
3. Concentrate purified protein sample
4. Visualize MAX-6xHis purity
5. Measure MAX-6xHis concentration



Let’s review the protein purification steps…

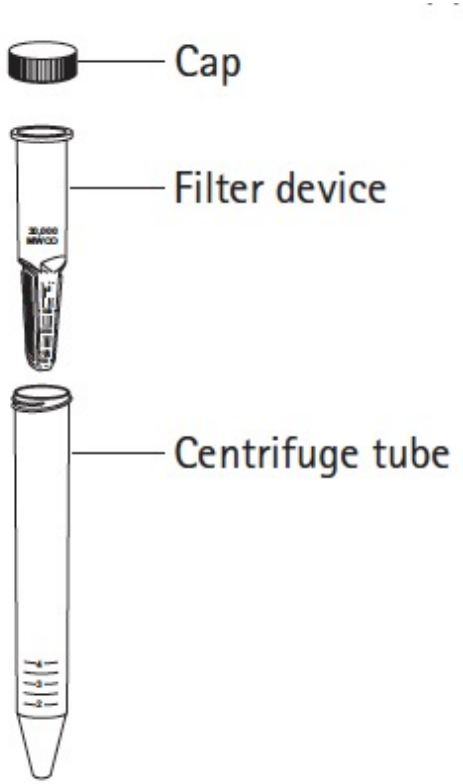
- Added lysonase – to what? why? *Added DNaseI – to what? why?*
- Centrifuged – what? why?
- Incubated with nickel resin – why?
- Washed with low concentration imidazole – why?
- Eluted with high concentration imidazole – why?

Overview of Mod 1 experiments:



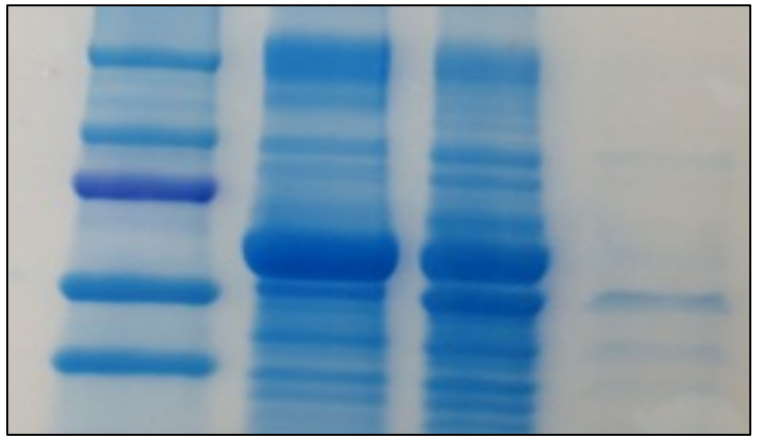
First, you will concentrate purified protein

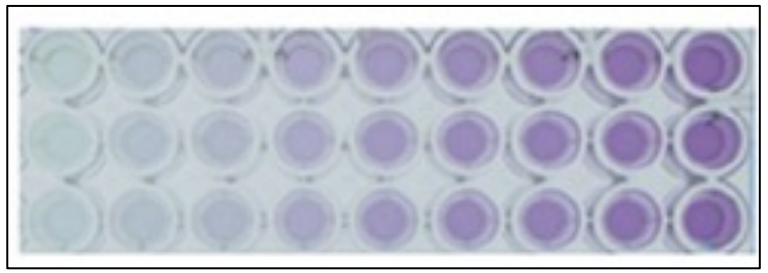
- Filter device sits within centrifuge tube…add protein to filter device for centrifugation
- Filter device has MW cutoff of 10 kDa …protein is retained in the filter device during centrifugation
- How does this concentrate the protein?



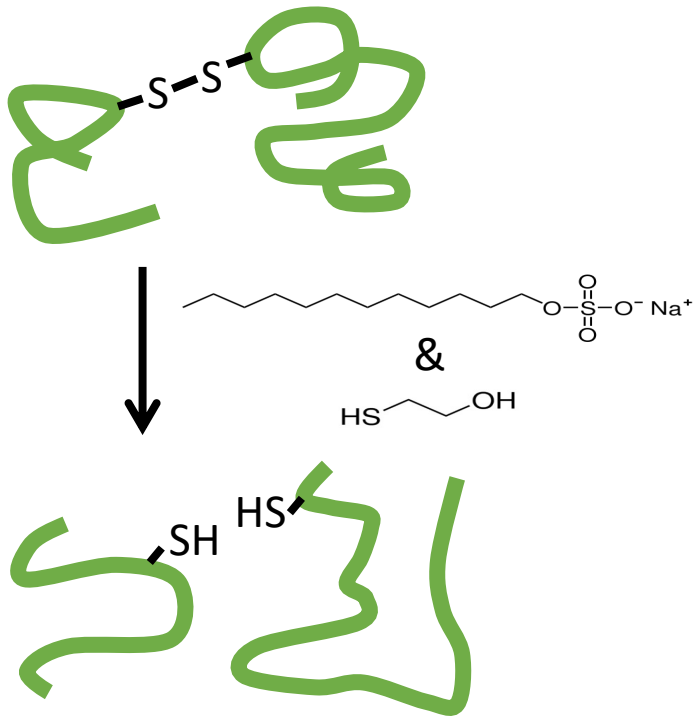
How will you assess purity and concentration?

- Check purity using SDS-PAGE
  - Visual detection of all proteins in sample
  - Used to assess purity / quality
- Measure concentration using BCA assay
  - Quantitative measure of all proteins in the sample
  - Used to calculate concentration





Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

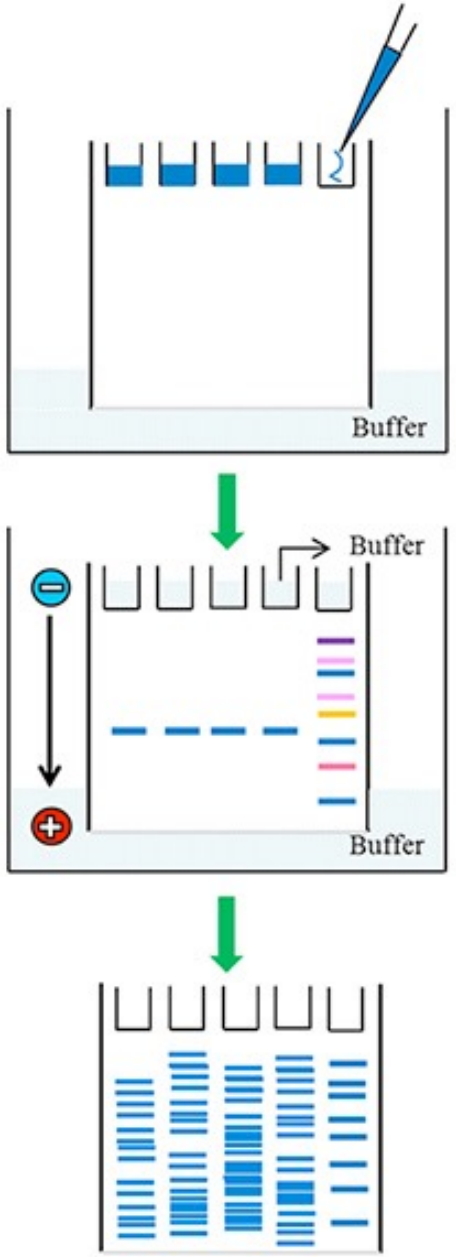


- Laemmli sample buffer (loading dye):
  - SDS
  - β-mercaptoethanol (BME)
  - bromophenol blue
  - glycerol
- Boiling:

How are proteins separated?

- Electrophoresis completed in TGS buffer
  - Tris-HCl
  - SDS
  - Glycine

- How does adding Laemmli buffer and boiling change proteins?
- What determines how far a protein migrates in the gel?



Be mindful when loading protein samples!

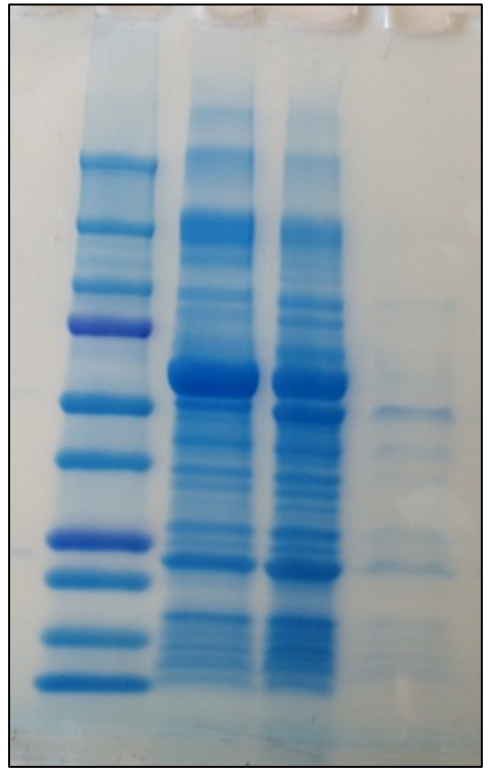
- Consider the order of your samples:
  - Pellet
  - Lysate
  - Flowthrough
  - Wash
  - Concentrated MAX-6xHis
  - Slurry
  - Ladder

- Figure for these results will be included in your Data summary!

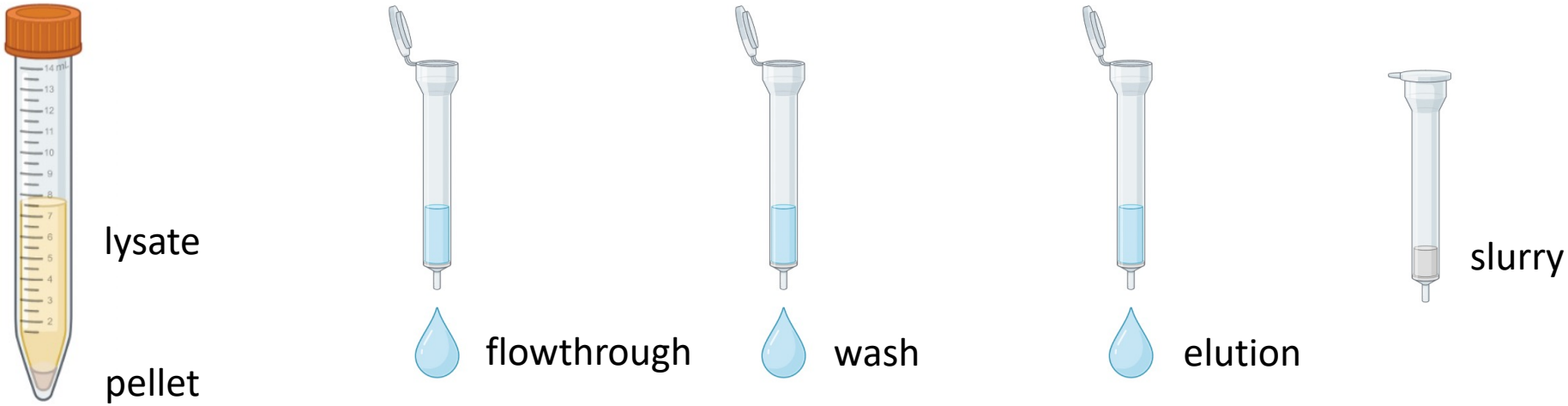
How are proteins visualized?

- Coomassie dye used to stain protein bands
  - Hydrophobic and electrostatic interactions with basic residues
  - Arg (also His, Lys, Phe, Trp)

- How will you know which band corresponds to MAX-6xHis?
- How does SDS-PAGE provide information regarding the purity of your protein sample?



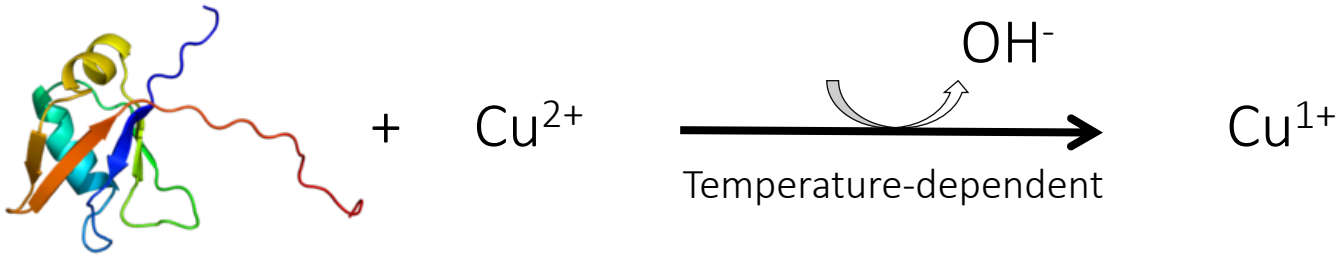
What are the expected results?



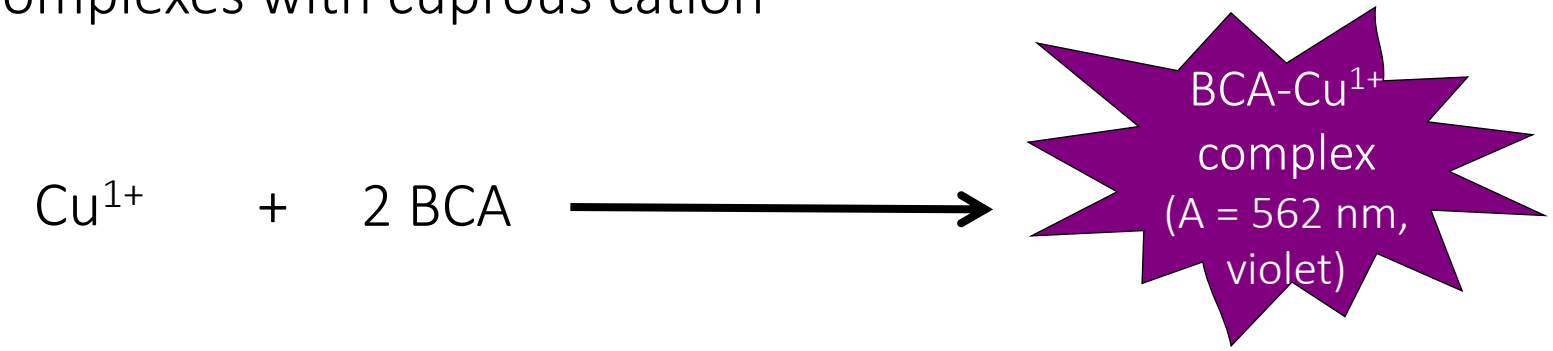
- Where do you / do you not expect to see MAX-6xHis? Why / why not?
- Where do you expect to see other cellular proteins?
- How will the elution sample differ from the concentrated protein sample?

Concentration: Bicinchoninic acid (BCA) protein assay

Step 1: Biuret reaction; chelation of copper with protein, reduction of copper

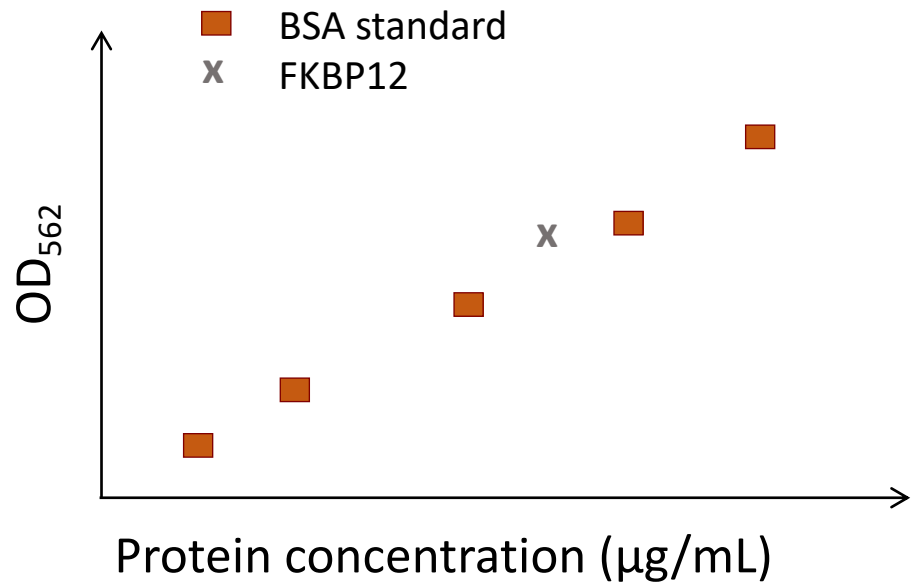


Step 2: BCA complexes with cuprous cation



BCA/Cu^{1+} absorbance proportional to protein concentration

- Standard curve generated using serial dilutions of bovine serum albumin (BSA)
  - Use fresh tips between tubes
  - Mix well between dilutions
  - Be sure to add correct volumes



- Is the calculated concentration an accurate measure of the amount of MAX-6xHis in your sample?

For today…

- Divide experiments between partners to ensure work is completed during class time
  - Be sure to share data / results!

For M1D4…

- Read journal article for in-class discussion
- *Draft outline of Background & Motivation for bonus feedback*