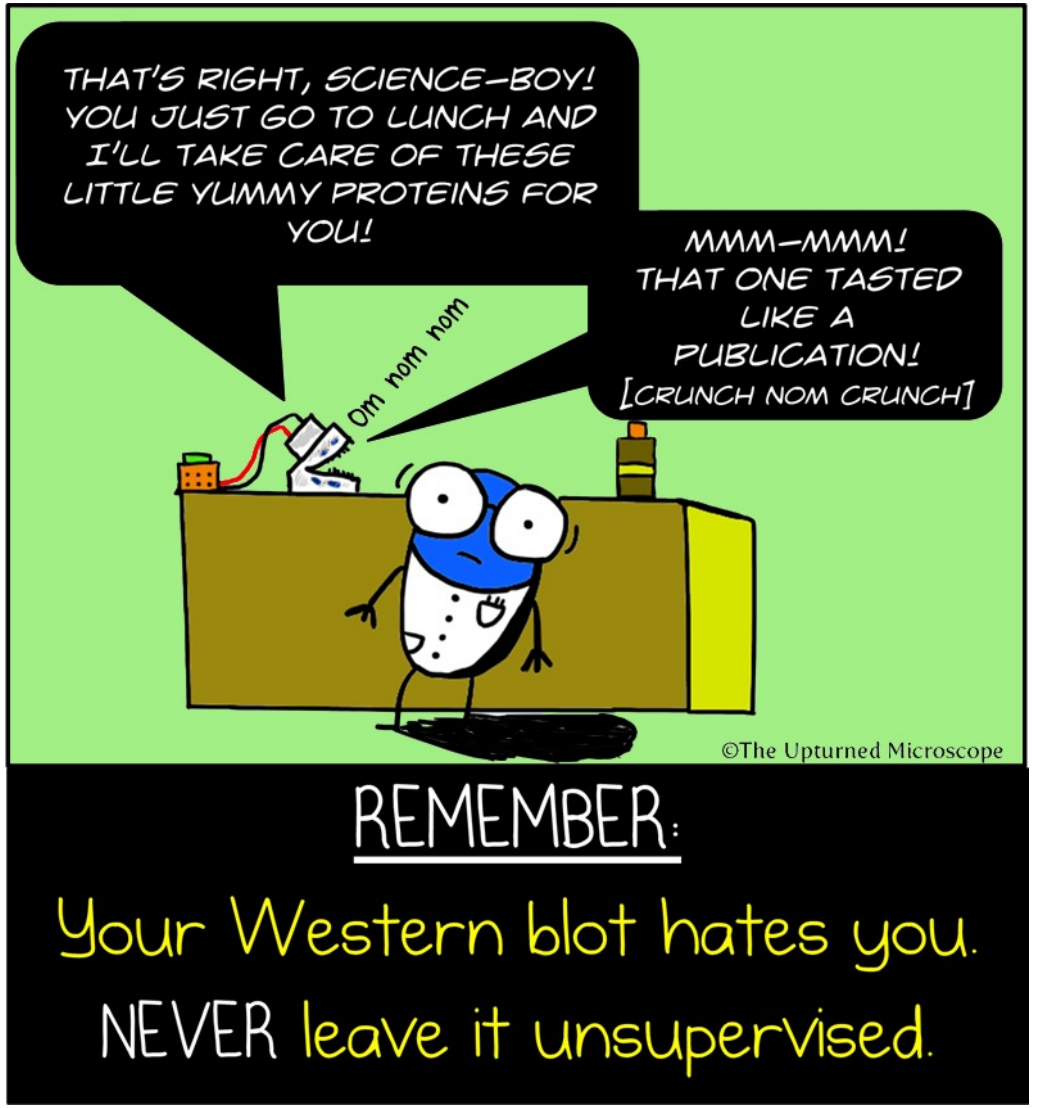
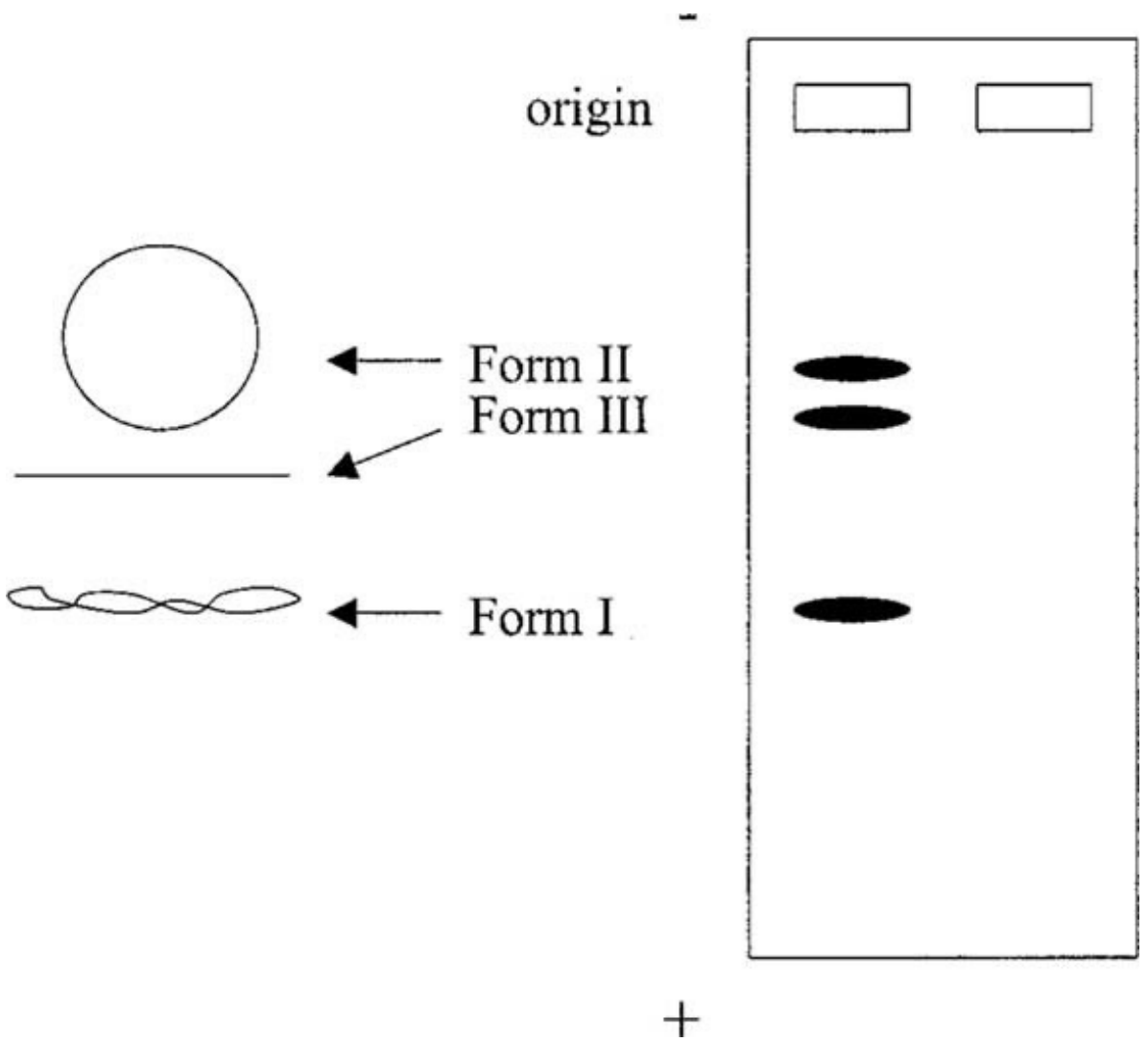
M2D3: Assess purity and concentration of purified protein

1. Prelab discussion
2. Visualize protein purity with SDS-PAGE
3. Measure protein concentration with BCA assay



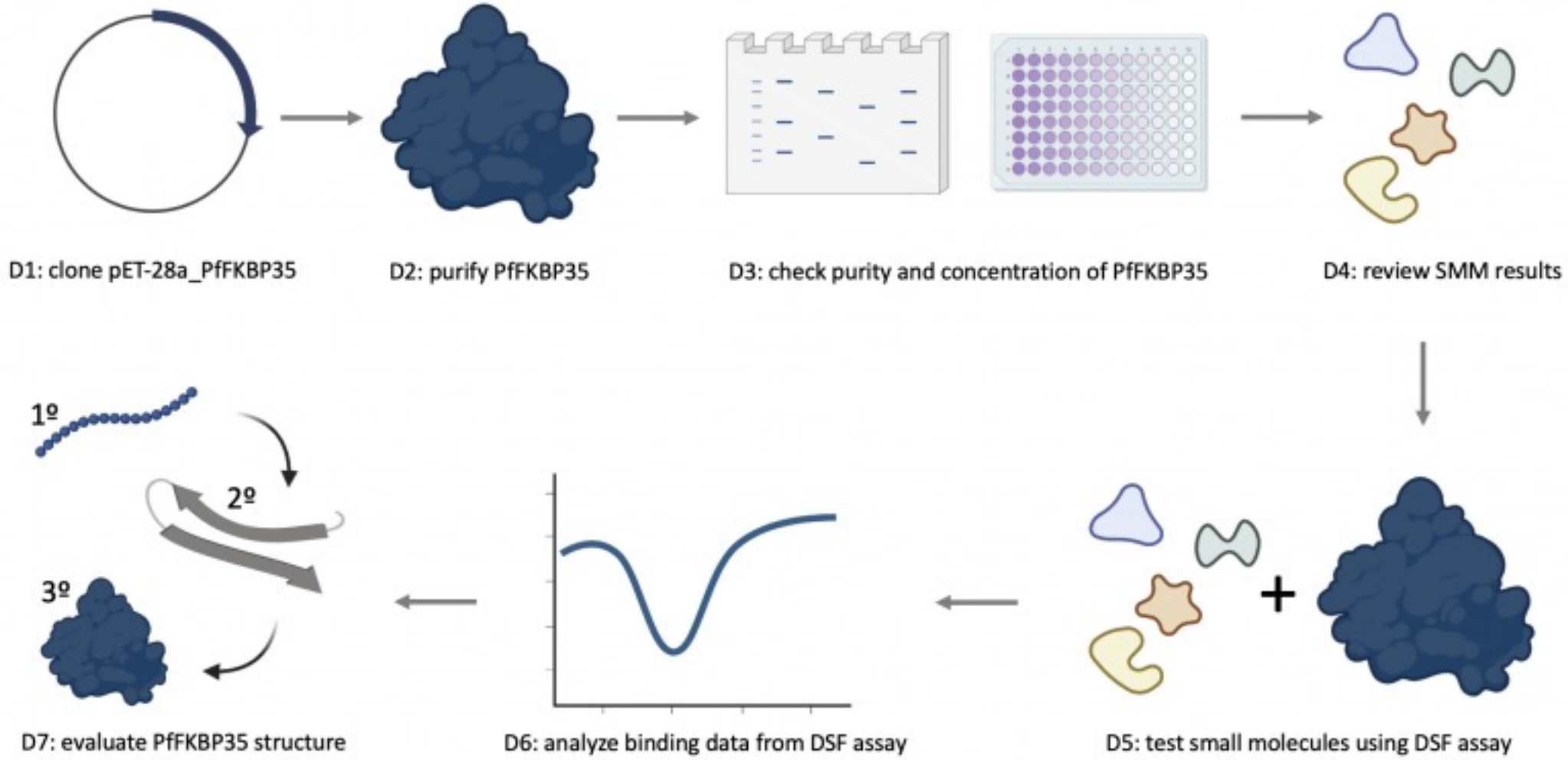
Notes on plasmid DNA on an agarose gel



De Matteos et al. 2006

Overview of M2: drug discovery

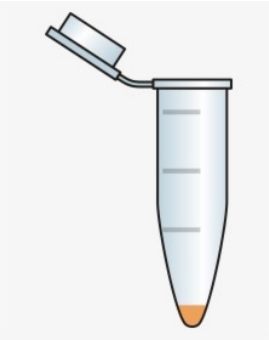
Research goal: Test small molecules for binding to the *Plasmodium falciparum* FKBP35 protein using a functional assay.



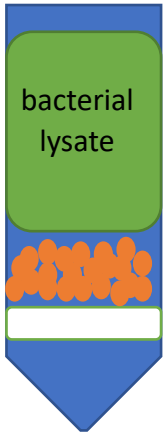
Protein purification review

- Why this step?

Pellet



Lysate

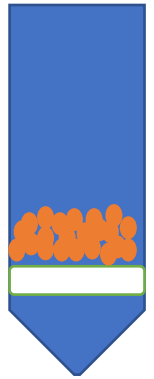


Flowthrough

Wash

Elution

Slurry



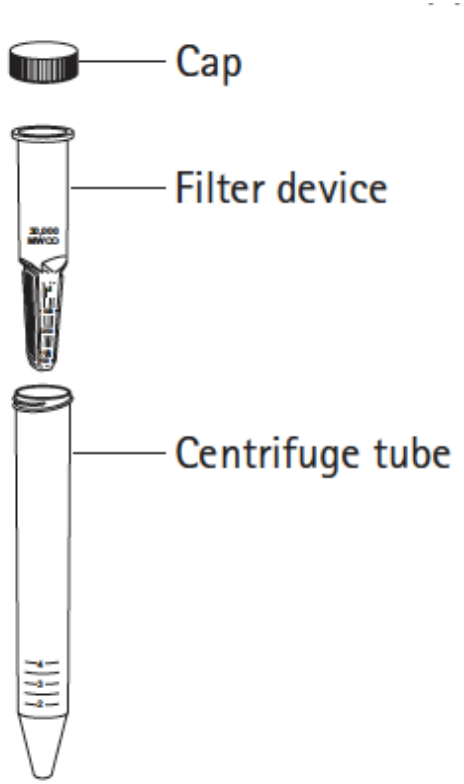
- What's on the resin?
- What's in the expelled liquid?

Concentrate protein before testing

- Filter device sits within centrifuge tube…add protein to filter device for centrifugation

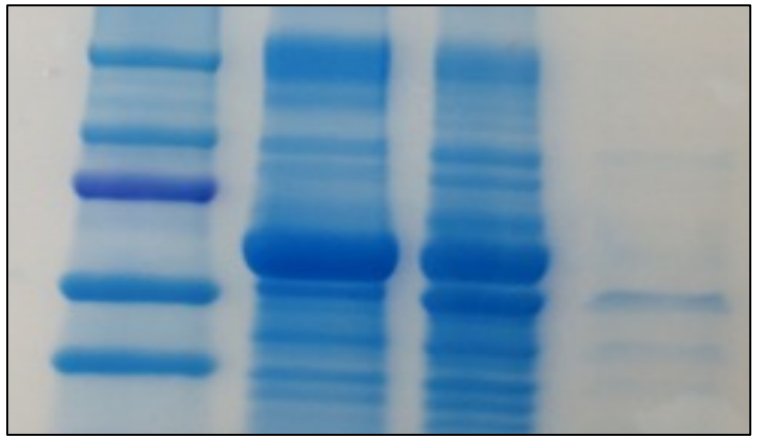
- Filter device has MW cutoff of 10 kDa …protein is retained in the filter device during centrifugation
  - **PfFKBP35 = 35kDa**
  - **His-tag = 2kDa**

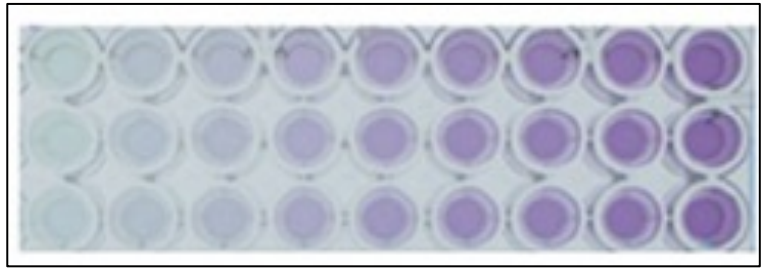
- How does this concentrate the protein?
- How does this remove excess biotin?



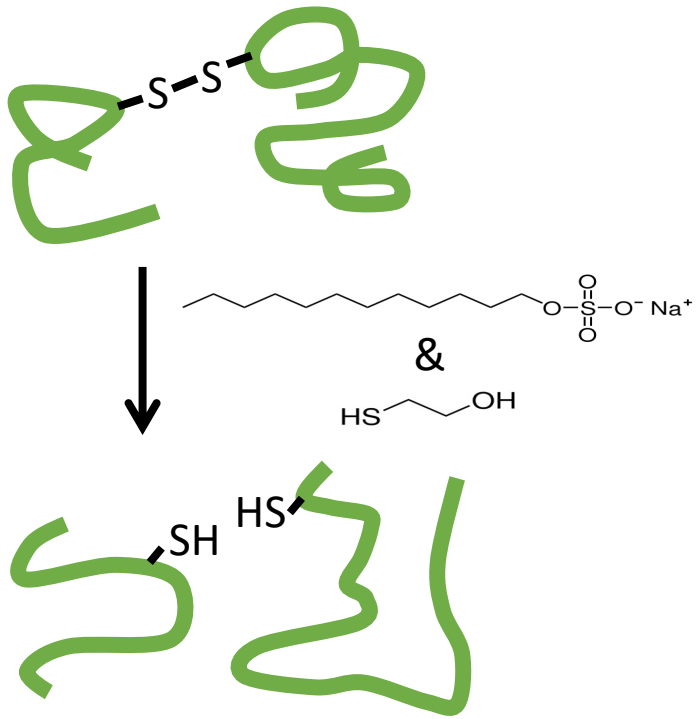
How will you assess purity and concentration?

- Check purity using SDS-PAGE
  - Visual detection of other proteins in sample
  - Identifies purity of sample at multiple stages of purification
- Measure concentration using BCA assay
  - Colorimetric assay
  - Calculate concentration from standard curve





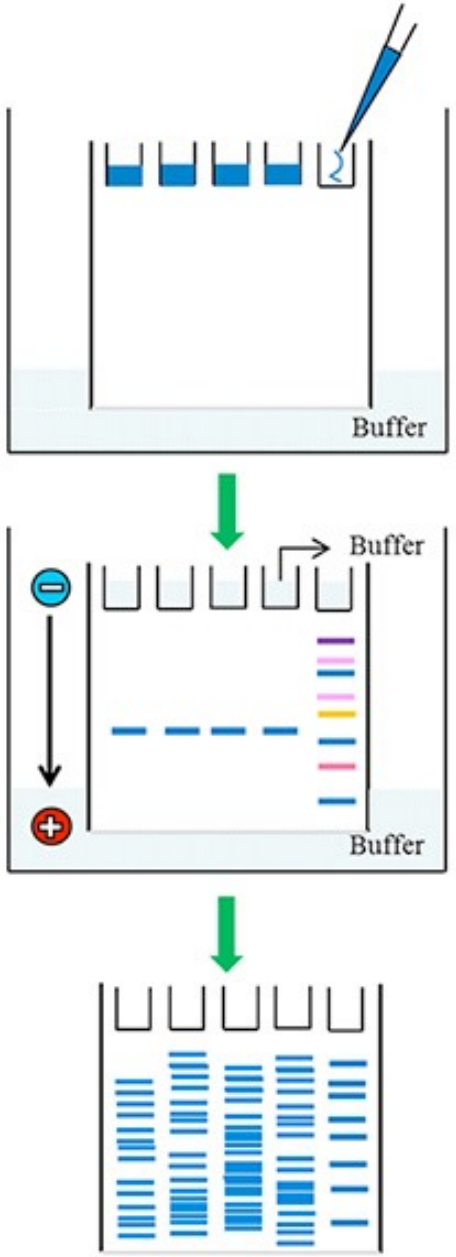
Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



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

How are proteins separated?

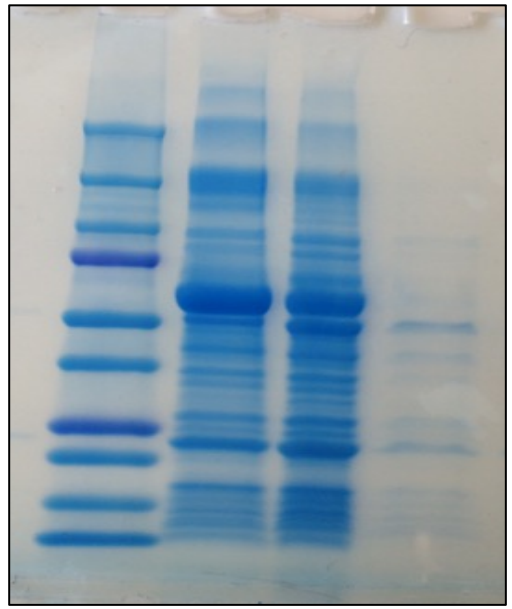
- Laemmli buffer and boiling results in _____ and _____ charged proteins
- SDS-PAGE separates proteins by _____
- Electrophoresis completed in TGS buffer
  - Tris-HCl
  - SDS
  - Glycine



How are proteins visualized?

Coomassie brilliant blue G-250 dye used to stain gel after electrophoresis

- Red if unbound (cationic form)
- Blue if bound to protein (anionic form)
- Hydrophobic and electrostatic interactions with basic residues
  - Arg (also His, Lys, Phe, Trp)



Be mindful when assessing SDS-PAGE protein samples

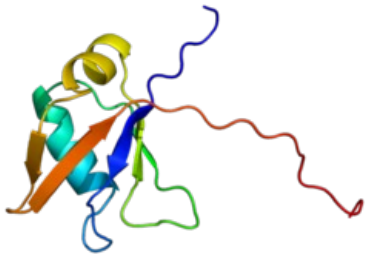
Consider the order of your samples:

1. molecular weight ladder
2. pellet
3. lysate
4. flow-through
5. wash
6. elution
7. resin
8. concentrated protein.

- Figure will be included in your Research Article!

Concentration: Bicinchoninic acid (BCA) protein assay

Step 1: Chelation of copper with protein, reduction of copper sulfate to copper ion



+ Cu^{2+} $\xrightarrow[\text{Temperature-dependent}]{OH^-}$ Cu^{1+}

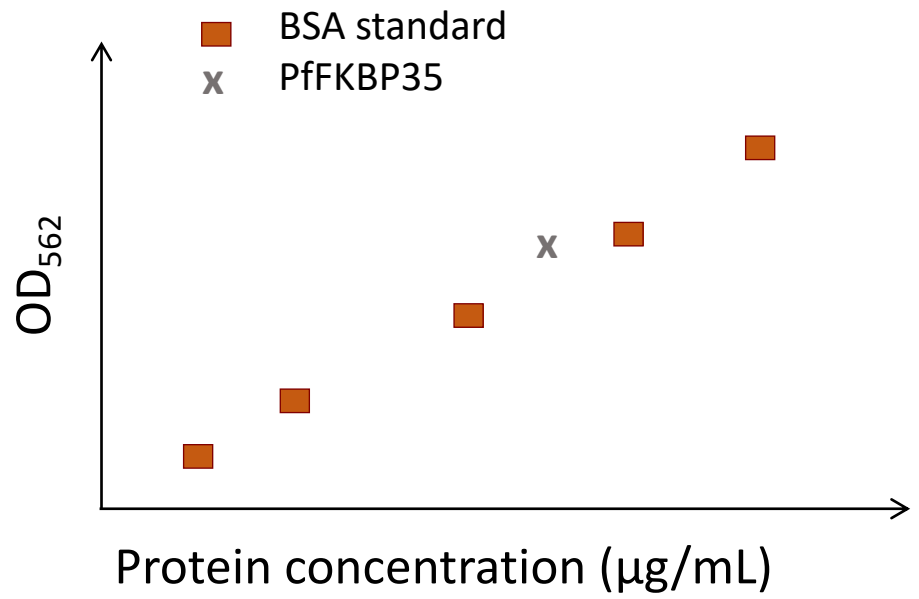
Step 2: BCA complexes with cuprous cation

Cu^{1+} + 2 BCA $\longrightarrow$ BCA-Cu^{1+} complex (A = 562 nm, violet)

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 mindful of volumes



For today…

- Complete the purity and concentration assessments
  - It’s good to divide the work load here!
  - Start immediately by putting your Elution into the concentration column to spin

For M2D4…

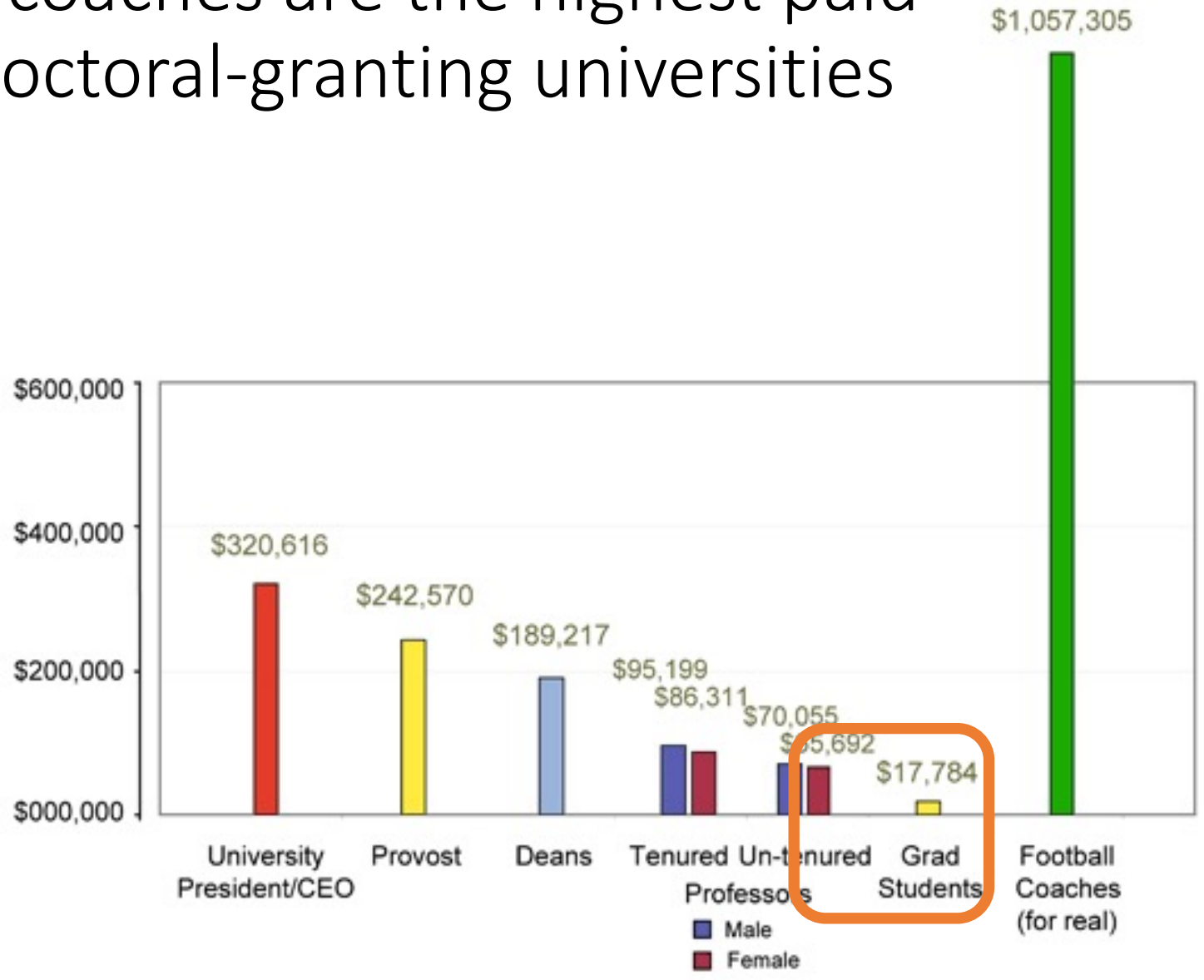
- Create a slide and write the accompanying script for your Journal Article presentation

Craft 1-2 slides using your journal article so you present key data from 1 figure

- Your slide(s) should show the data and highlight the key finding(s).
- The information should be clear and large enough to read.
- Keep text to a minimum. (NO figure captions on slide!)
- The title should state the take-home message of the data that are shown.

EXAMPLE SLIDE: Football coaches are the highest paid academic employees at doctoral-granting universities

- Data represent expression of Y using method A
- Possibly something about the control(s), if applicable
- Perhaps an important note about the data that is not already stated in the title
- Transition to next slide…



PhD Comics