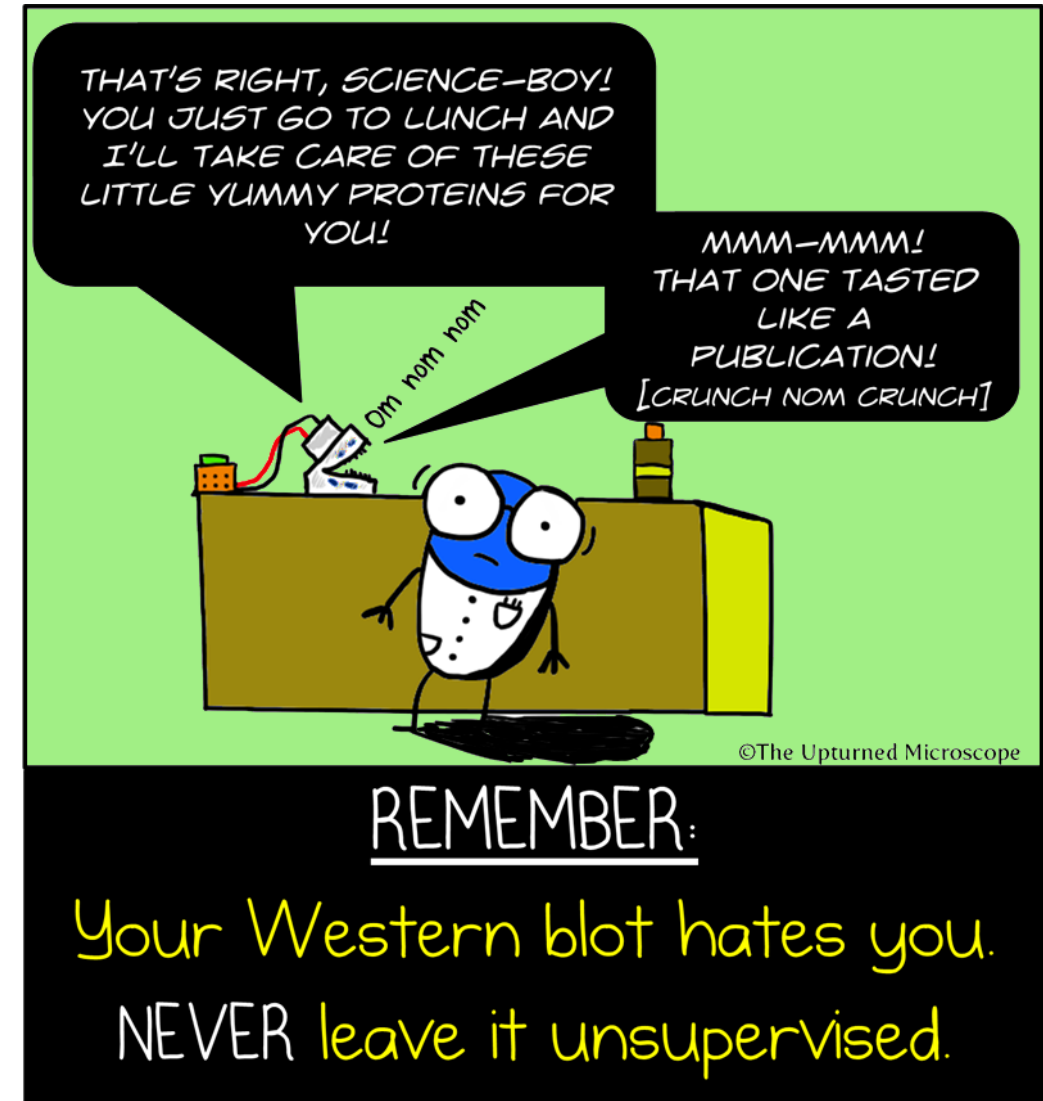
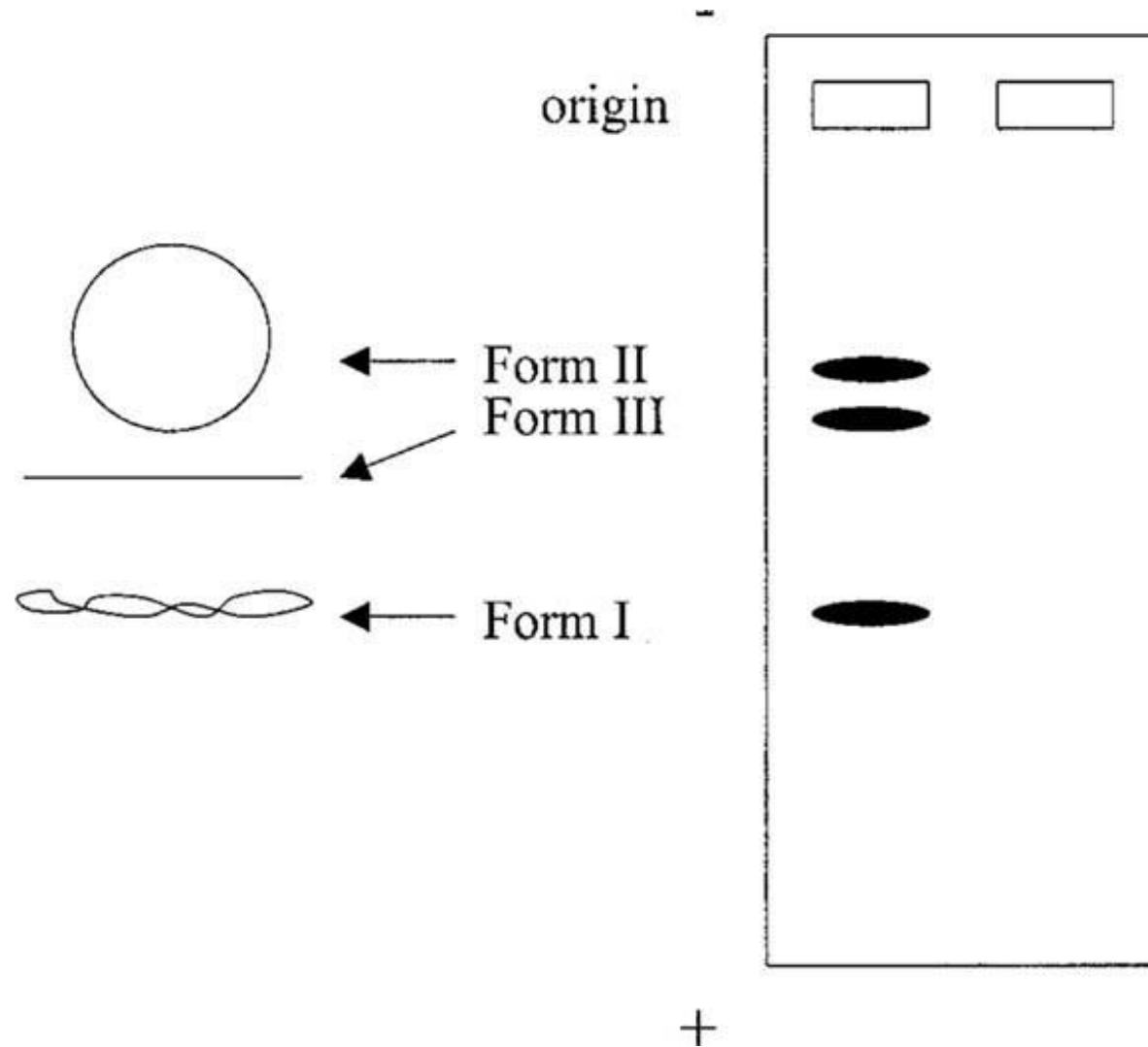


# M2D3: Assess purity and concentration of purified protein

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

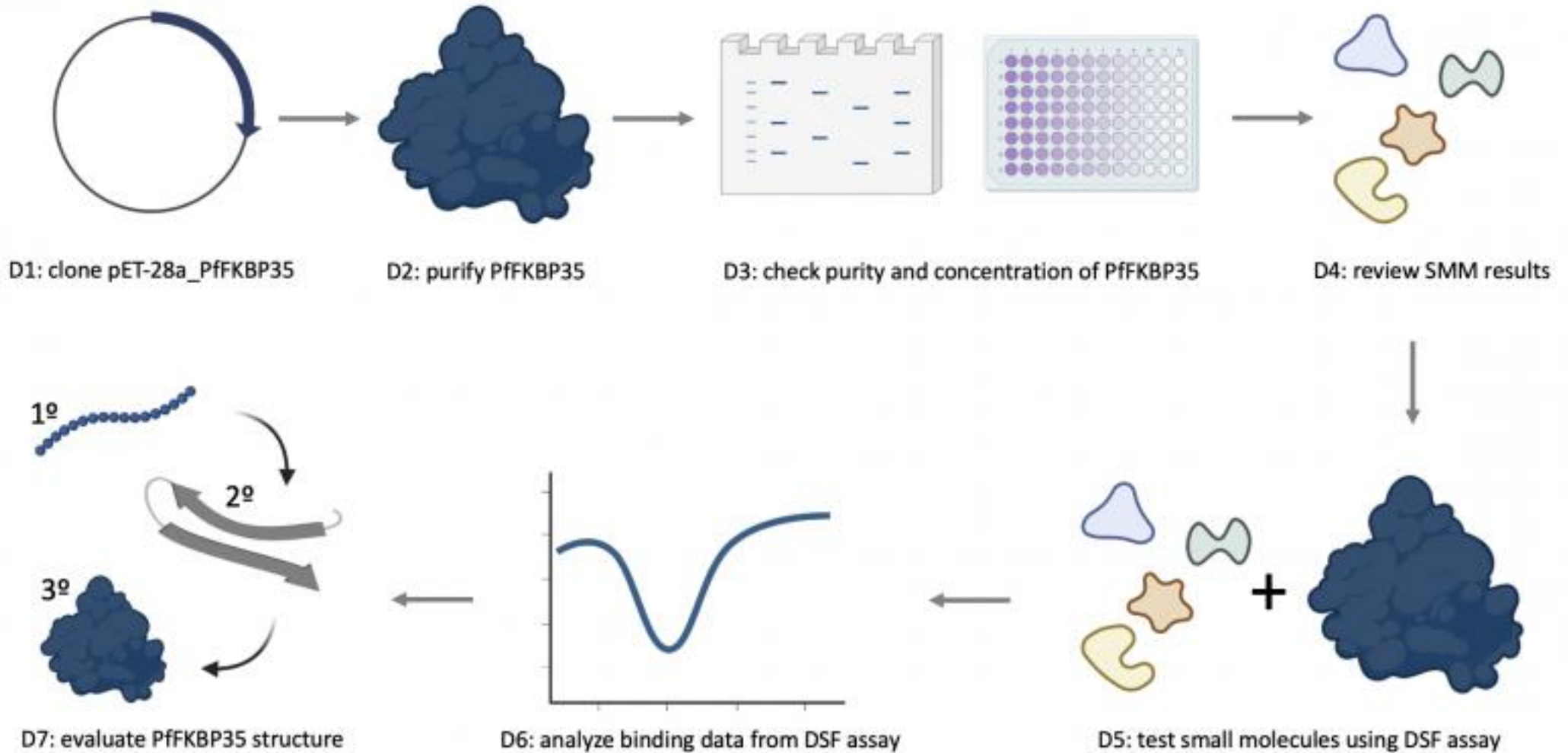


# Notes on plasmid DNA on an agarose gel



# Overview of M2: drug discovery

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

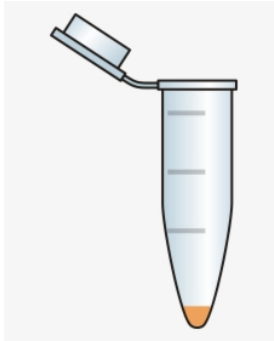


# Protein purification review

- Why this step?

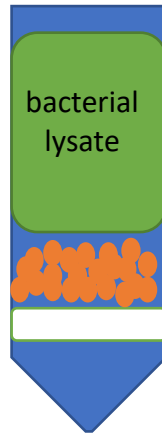
Soluble proteins,  
POI

## Pellet



Lipids, insolubles

## Lysate



## Flowthrough



Unbound soluble  
proteins

## Wash



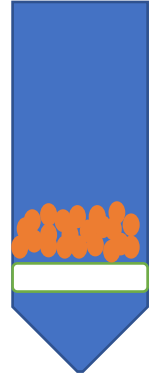
Weakly bound  
protein

## Elution



Protein of  
interest

## Slurry

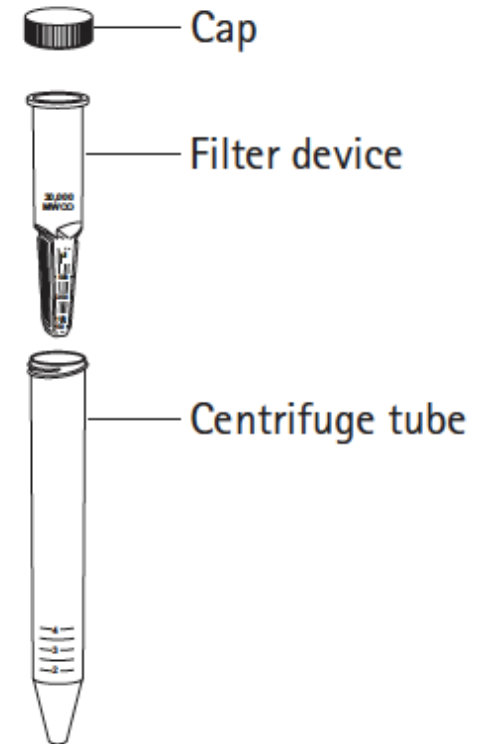


Ideally, nothing

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

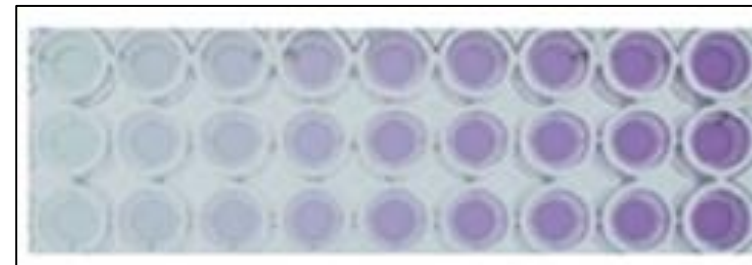
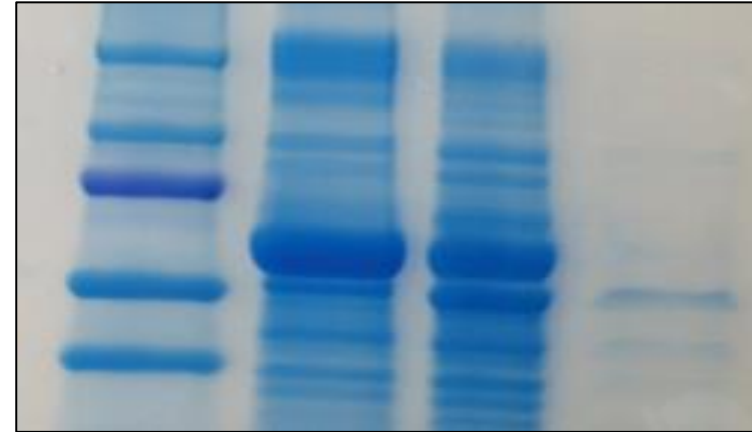
# Concentrate protein before testing

- 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 imidazole?

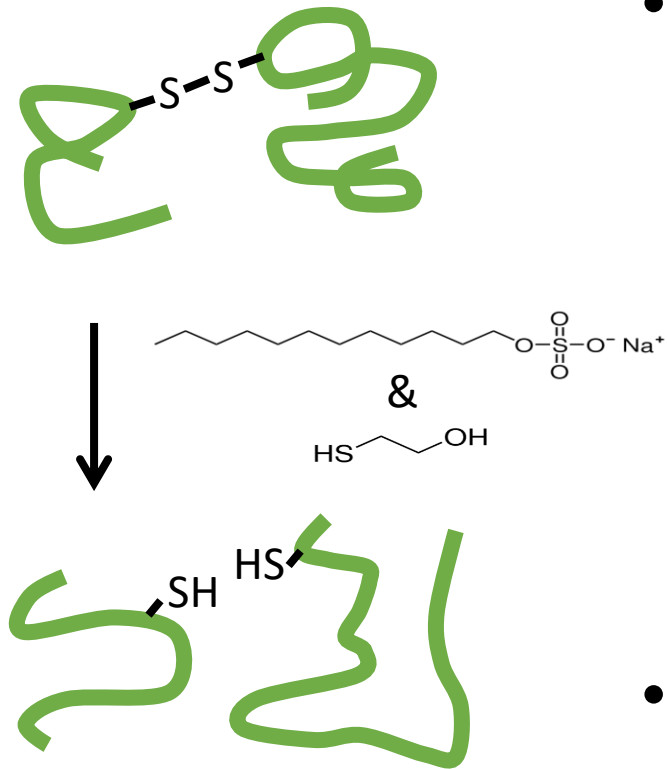
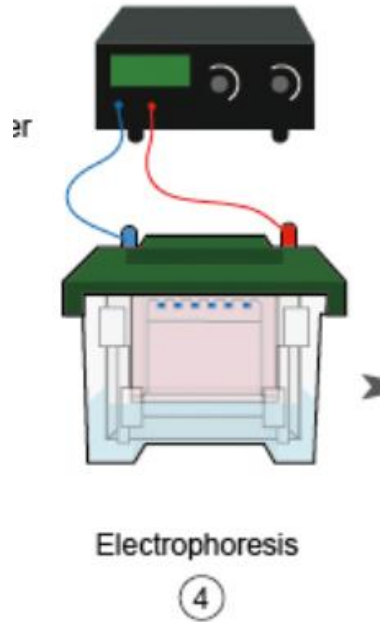


# How will you assess purity and concentration?

- 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



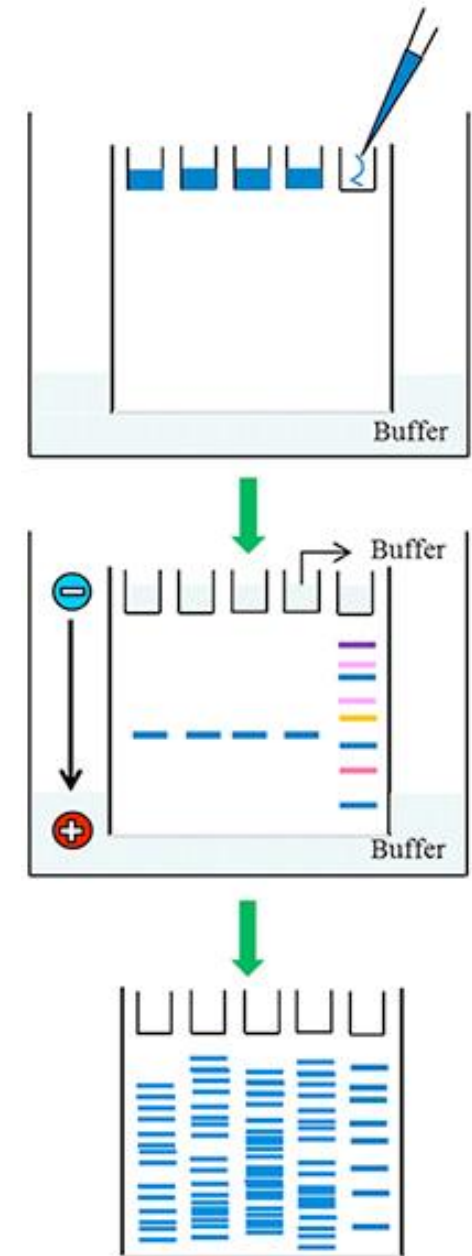
# Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



- Laemmli sample buffer / loading dye:
  - SDS
  - $\beta$ -mercaptoethanol (BME) **Breaks disulfide bonds**
  - bromophenol blue **Dye front**
  - glycerol **Weighs down the liquid sample**
- Boiling: **Ensures complete denaturation**

# How are proteins separated?

- Laemmli buffer and boiling results in linear and (-) charged proteins
- SDS-PAGE separates proteins by Size
- Electrophoresis completed in TGS buffer
  - Tris-HCl
  - SDS
  - Glycine

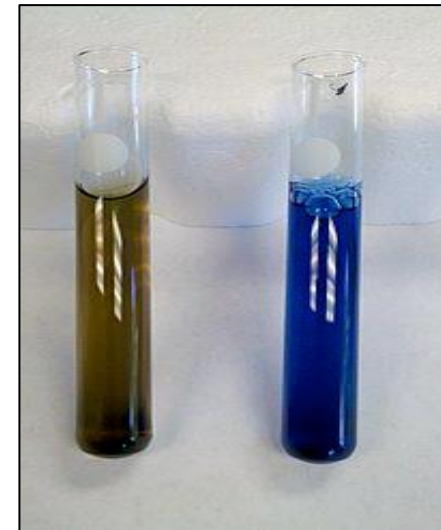
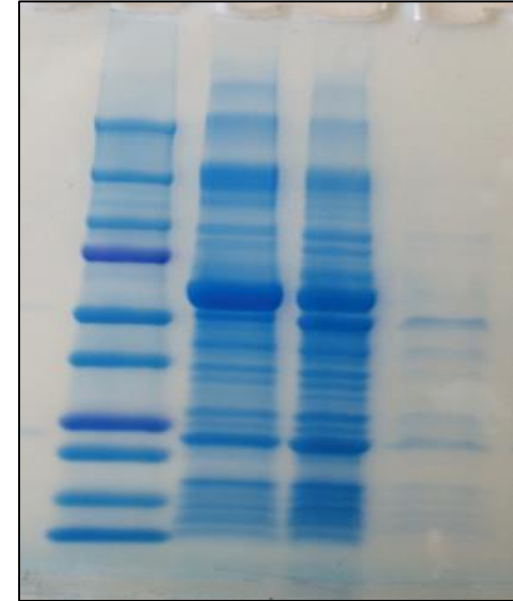




# How are proteins visualized?

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:

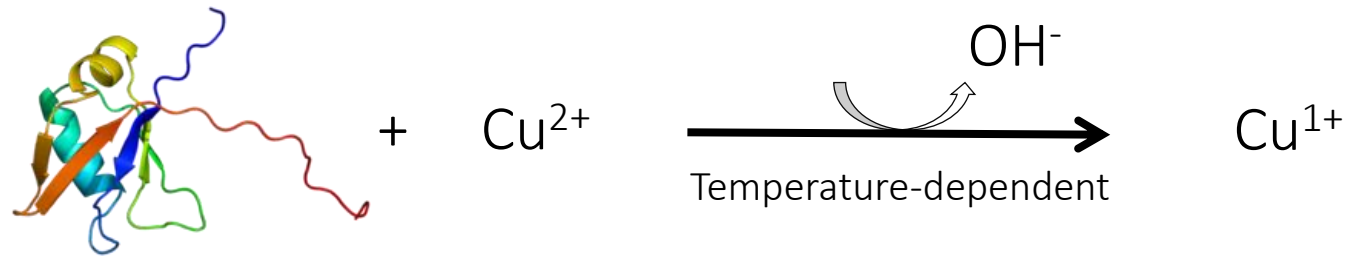
1. molecular weight ladder
2. pellet
3. lysate
4. flow-through
5. wash
6. elution
7. resin
8. concentrated protein.



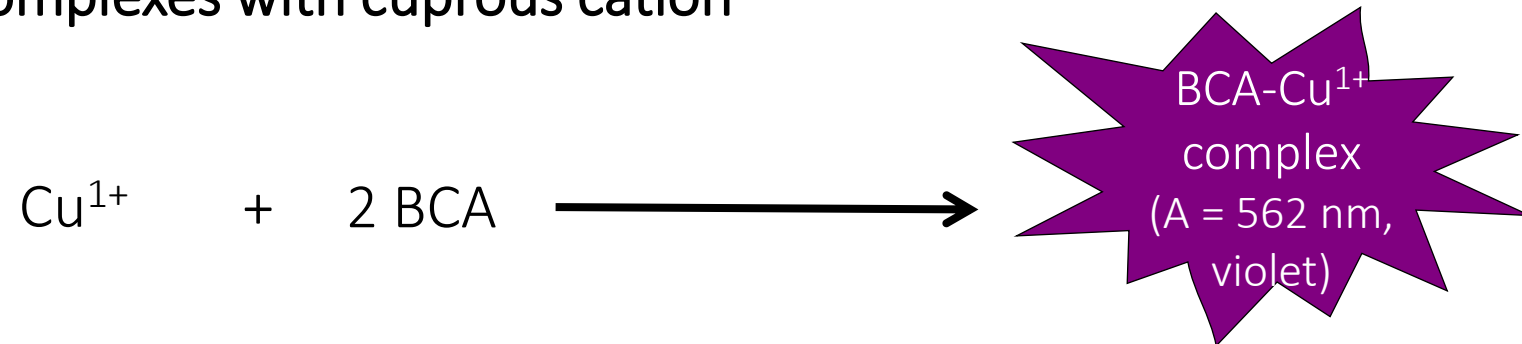
- Figure will be included in your Research Article!

# Concentration: Bicinchoninic acid (BCA) protein assay

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



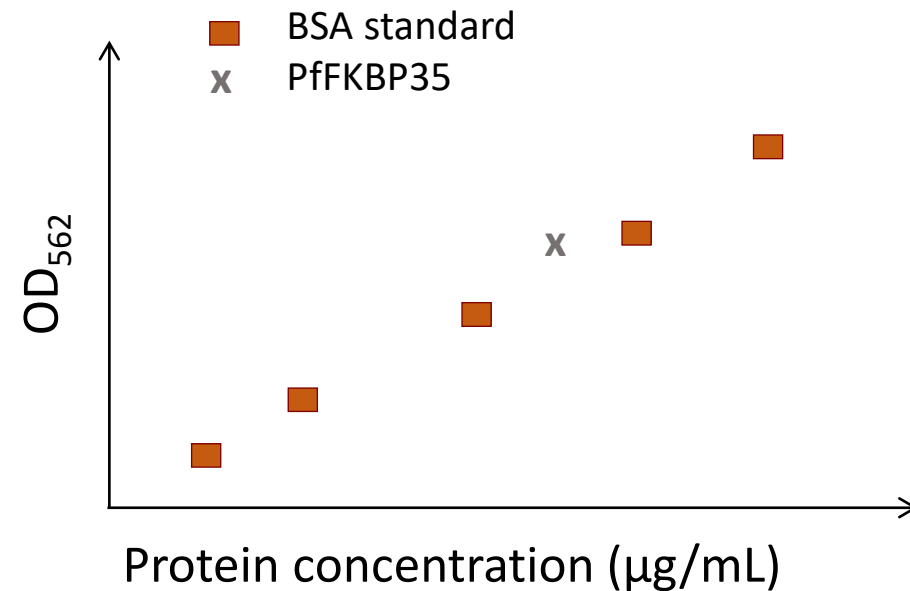
Step 2: BCA complexes with cuprous cation



# BCA/Cu<sup>1+</sup> 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...

