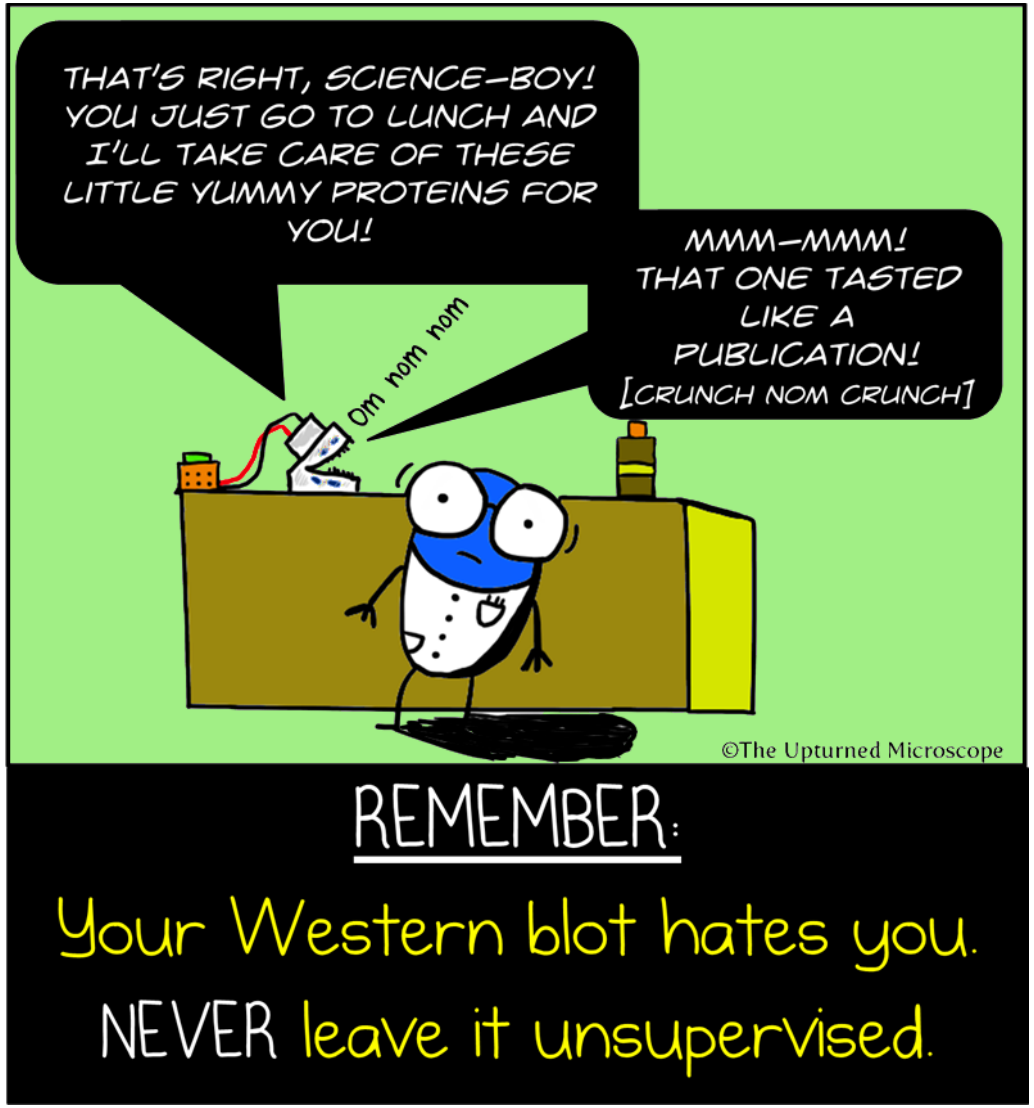
M2D3: Assess purity and concentration of purified protein

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

Extra Office Hours:
Tuesday/Wednesday 1-5pm



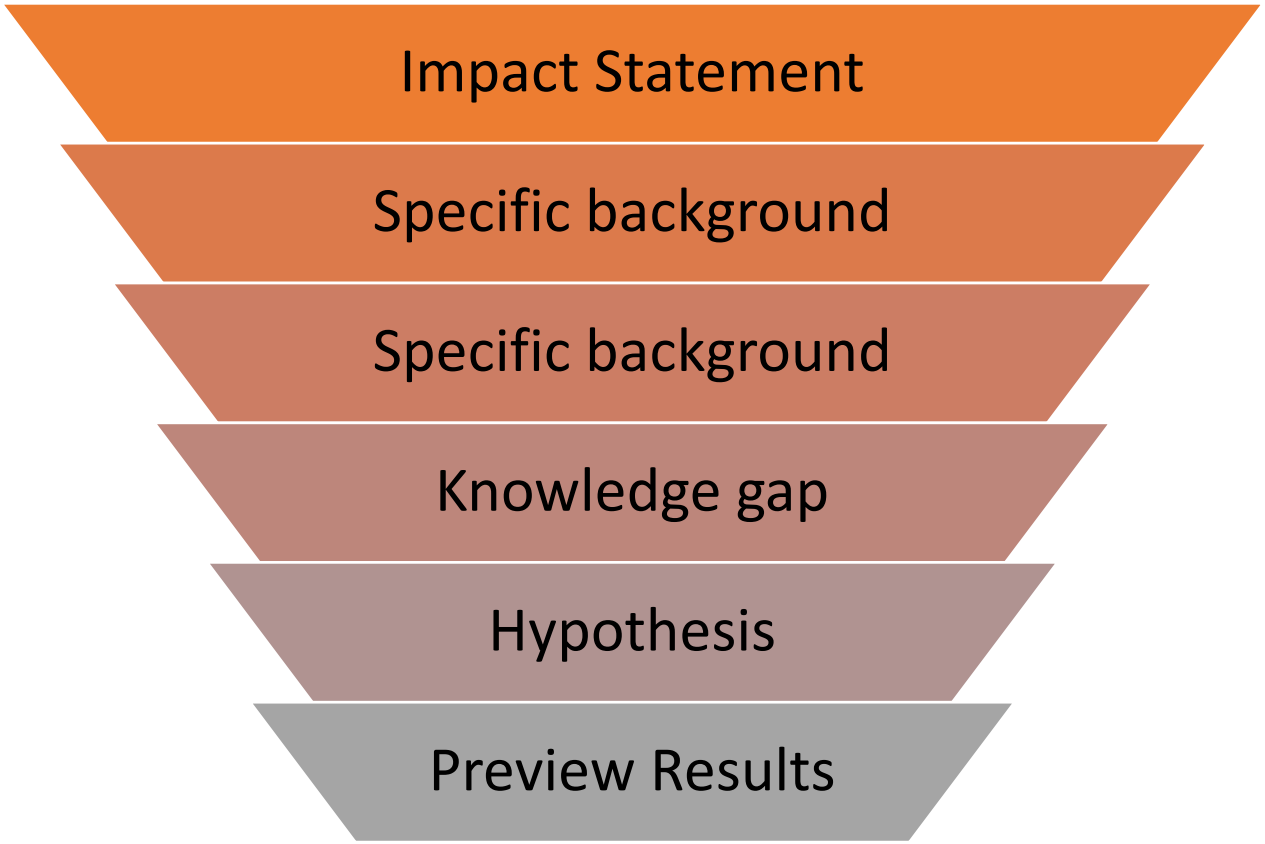
Homework

Outline the Introduction for your Research Article

Due M2D4: Tuesday, Oct 31!

Structure of the Introduction

- Looks suspiciously like the Background and Motivation from the Data Summary…



- **Broad context** for your work
- Why is this work **important**?
- What information from the **current literature** is needed to understand the work?
- What **gap** in the current literature will your project address?
- What is your research goal/**hypothesis**?
- **Here we show…**

- Make sure transitions from one topic to the next are clear

Broad outline example

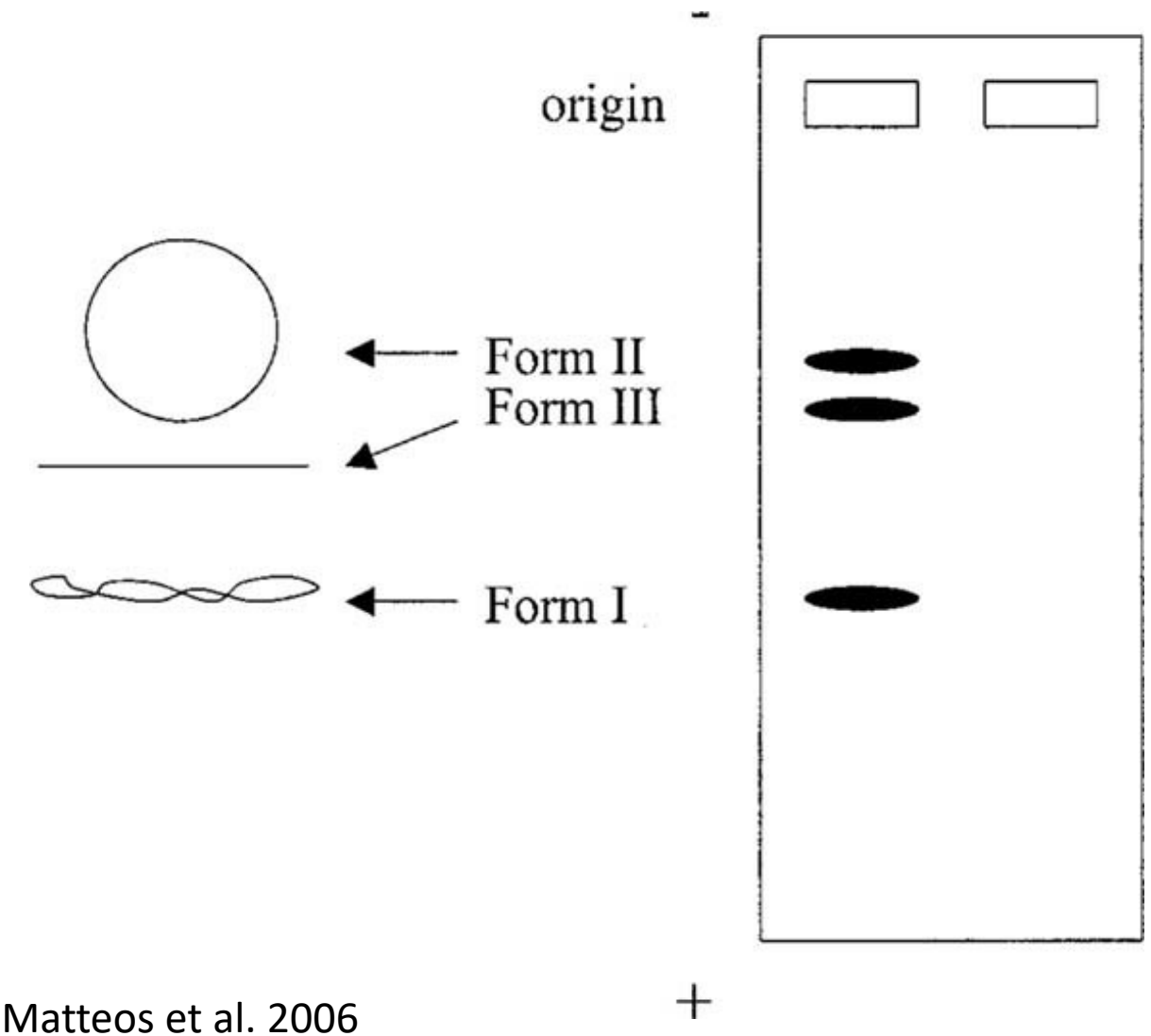
- **Impact statement**:
  - *Spinal cord injury (SCI) is bad*
- **Specific background**:
  - Concepts
    - *Why is this an important problem to solve?*
    - *What do I need to understand about SCI to follow this project?*
  - Components
    - *Introduce what we're working with (receptor and drug)*
  - Techniques (if relevant)
    - *Implant drug delivery system*
- **Knowledge gap**:
  - *Currently no effective treatments, perhaps because none are able to be given rapidly*
- **Research goal/Hypothesis**:
  - *Receptor-drug combination paired with the delivery system will encourage both axon growth from the injury site and functional recovery following injury*
- **Here we show**…
  - *We see axon growth through the tissue scar following injury*
  - *Functional range of motion is recovered*

Needs transitions!

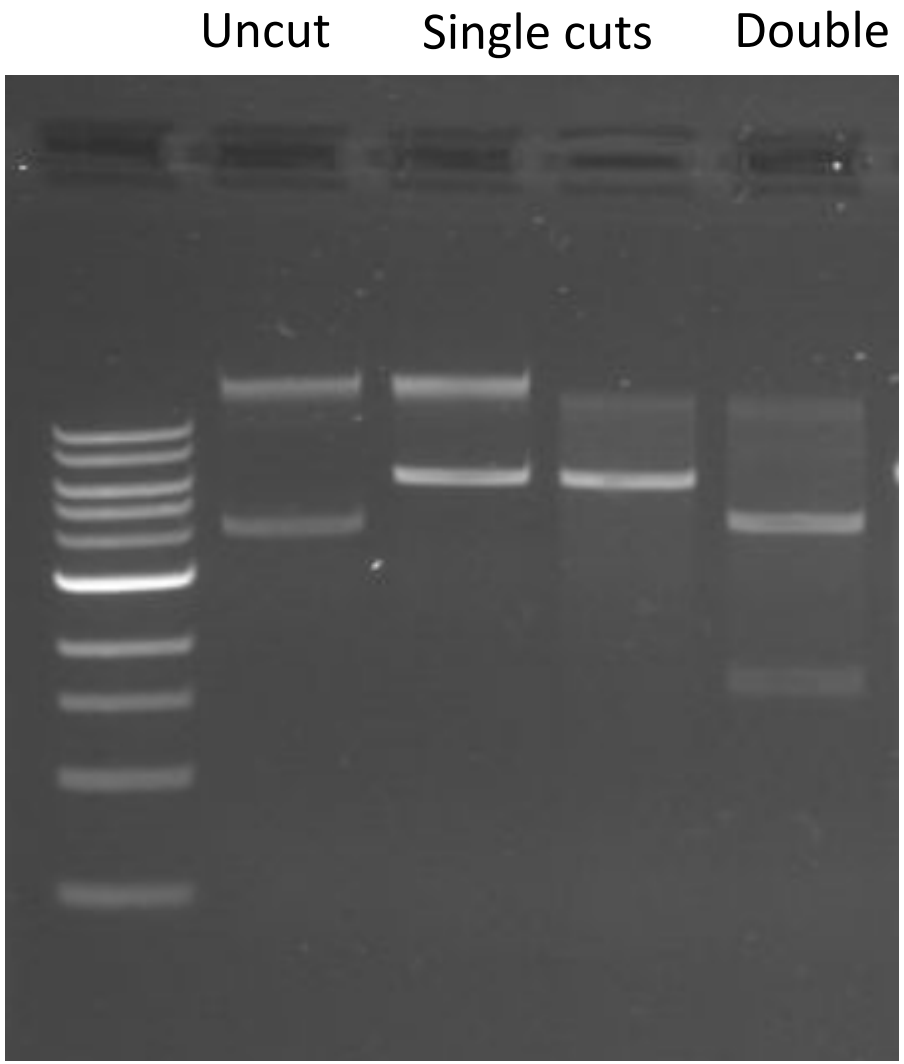
Lab work

SDS-PAGE gels and BCA assays

Notes on plasmid DNA on an agarose gel



De Matteos et al. 2006



Protein purification review

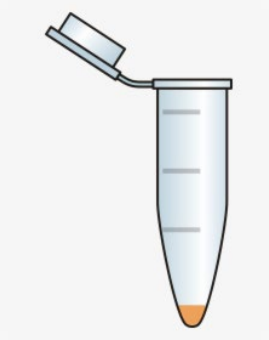
- What's happening?

Protein Binding

Low Imidazole

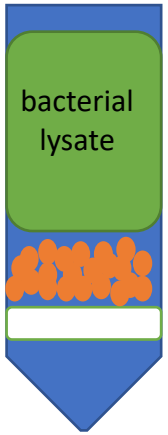
High Imidazole

Pellet



Lysis
- Debris
- Insoluble stuff
- Lipids

Lysate



Flowthrough

POI + nonspecific binders

Non binders

Wash

POI

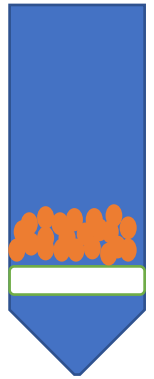
Nonspecific Binders

Elution

POI

No POI remaining

Slurry



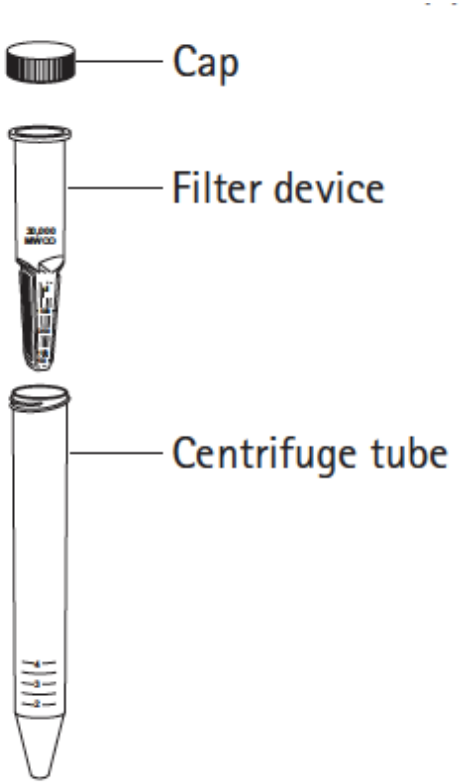
- What's on the resin?
- What's in the expelled liquid?

Concentrate protein before testing

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

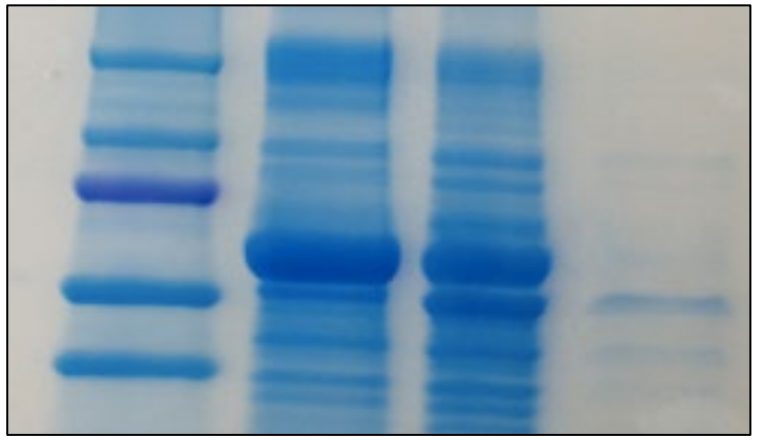
- Filter device has MW cutoff of 10 kDa …protein is retained in the filter device during centrifugation
  - **PfFKBP35 = 35kDa**
  - **His-tag = 2kDa**

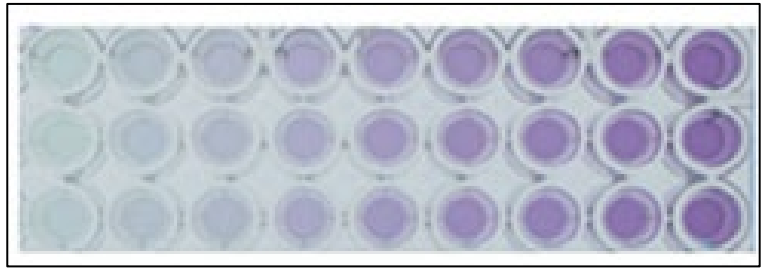
- How does this concentrate the protein?
- How does this remove excess imidazole?



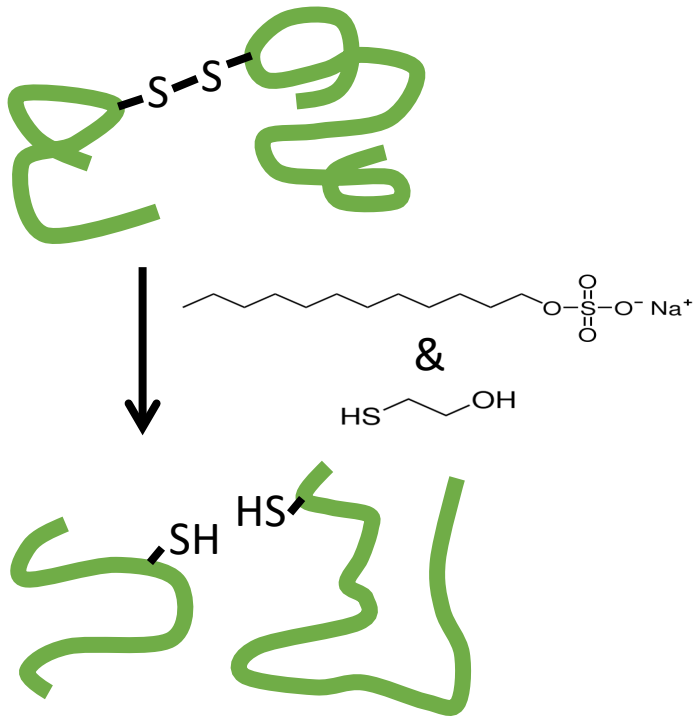
How will you assess purity and concentration?

- 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





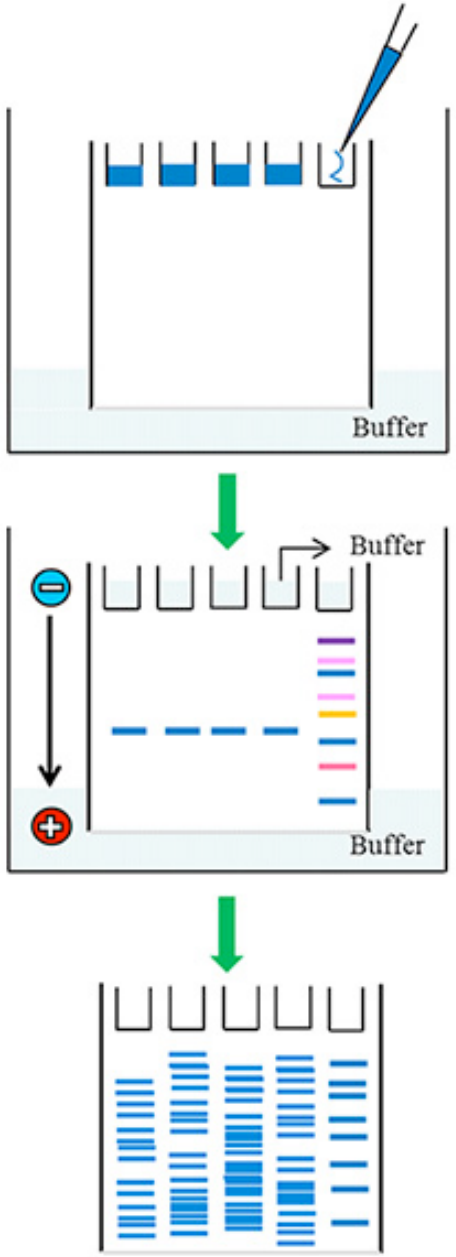
Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



- Laemmli sample buffer / loading dye:
  - SDS — Detergent that denatures proteins
  - β-mercaptoethanol (BME) — Breaks disulfide bonds
  - bromophenol blue — Visualizes samples in gel
  - glycerol — Weighs samples down
- Boiling: — Really, really trying to denature everything

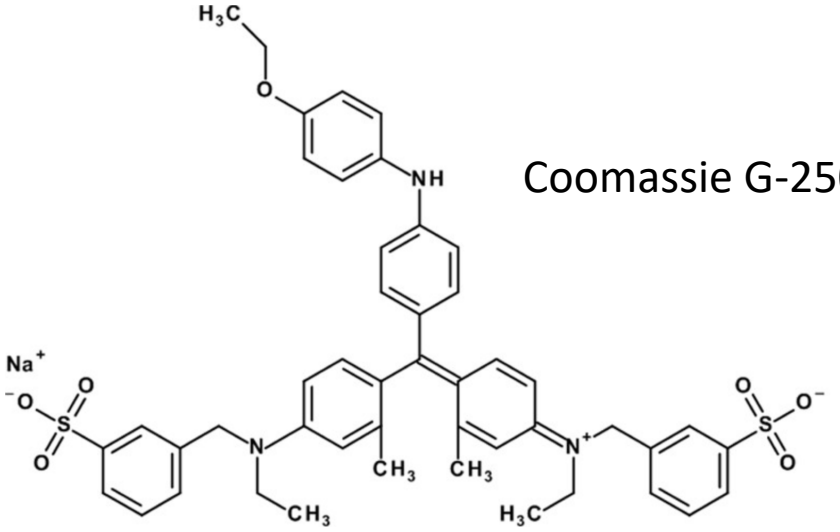
How are proteins separated?

- Laemmli buffer and boiling results in Denatured and negatively charged proteins
- SDS-PAGE separates proteins by size
- Electrophoresis completed in TGS (Running) buffer
  - Tris-HCl
  - SDS
  - Glycine



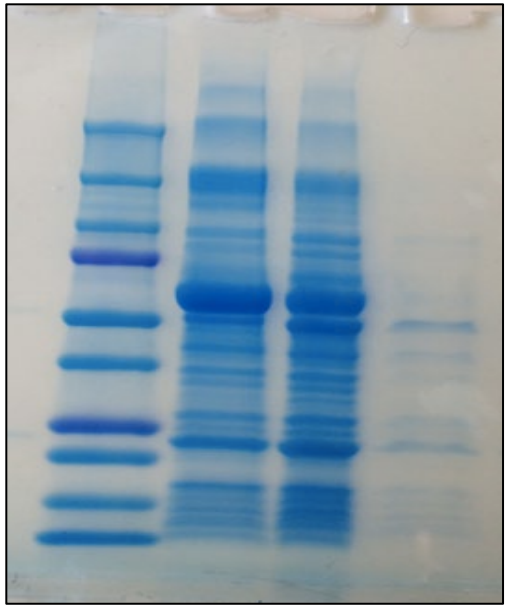
How are proteins visualized?

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



- Hydrophobic and electrostatic interactions with basic residues
  - Arg, His, Lys, Phe, Tyr, Trp

- Complex between dye and amino acids is blue
  - Useful to visualize protein on a gel



Be mindful when assessing SDS-PAGE protein samples

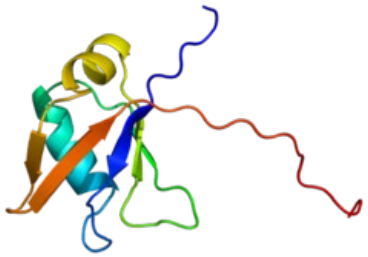
Consider the order of your samples & Record them:

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+}

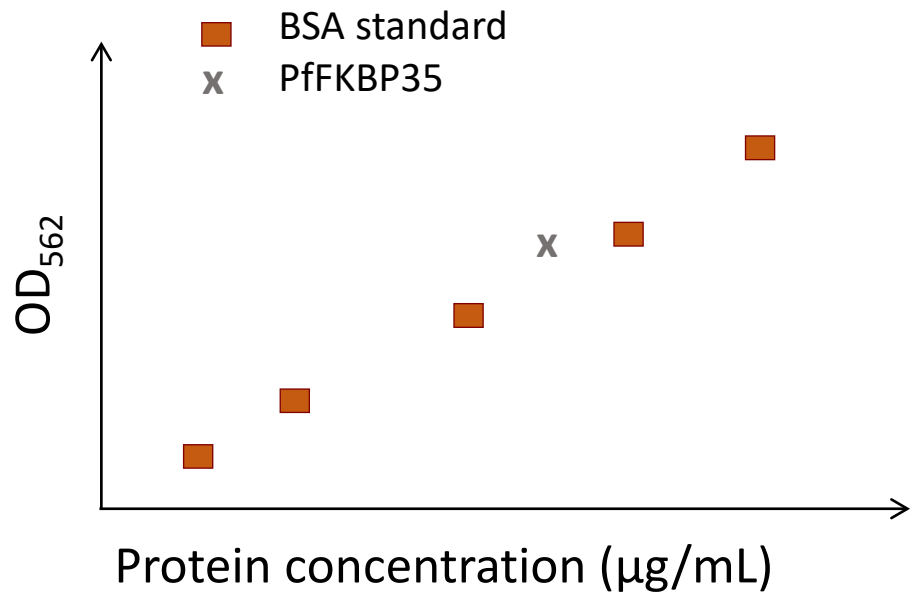
Step 2: BCA complexes with cuprous cation

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
  - Divide the work load here!
  - Start immediately by putting your Elution into the concentration column to spin