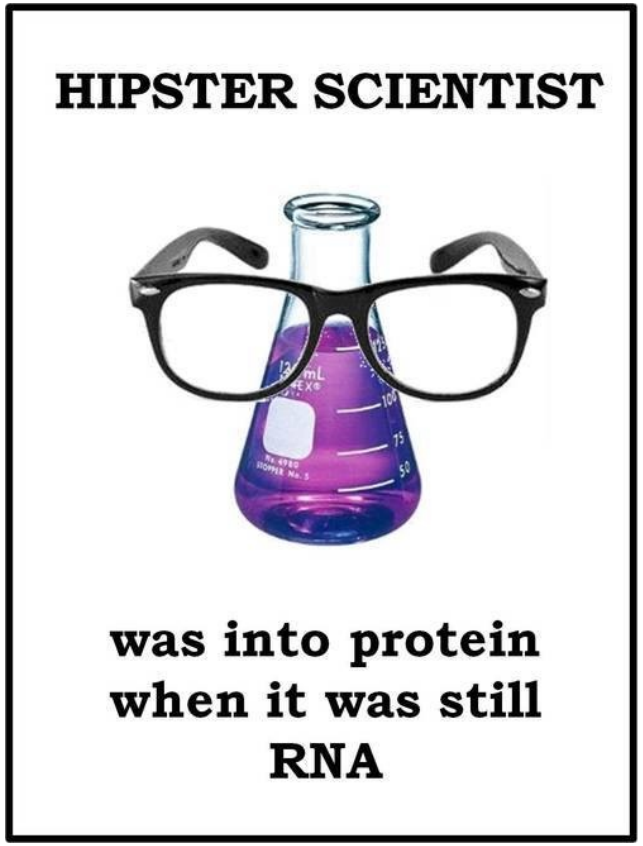
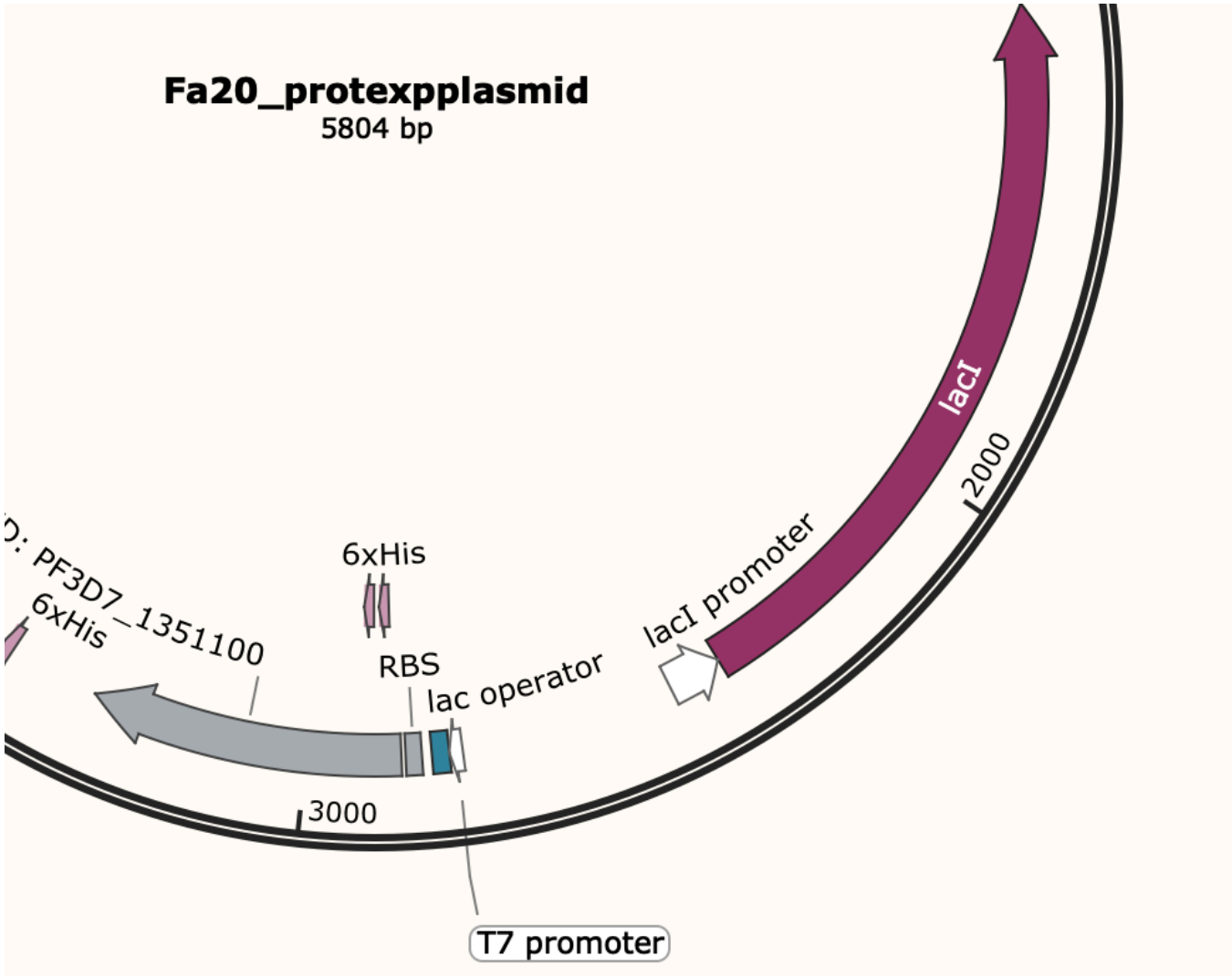
M2D3:
Assess purity and concentration of purified protein

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

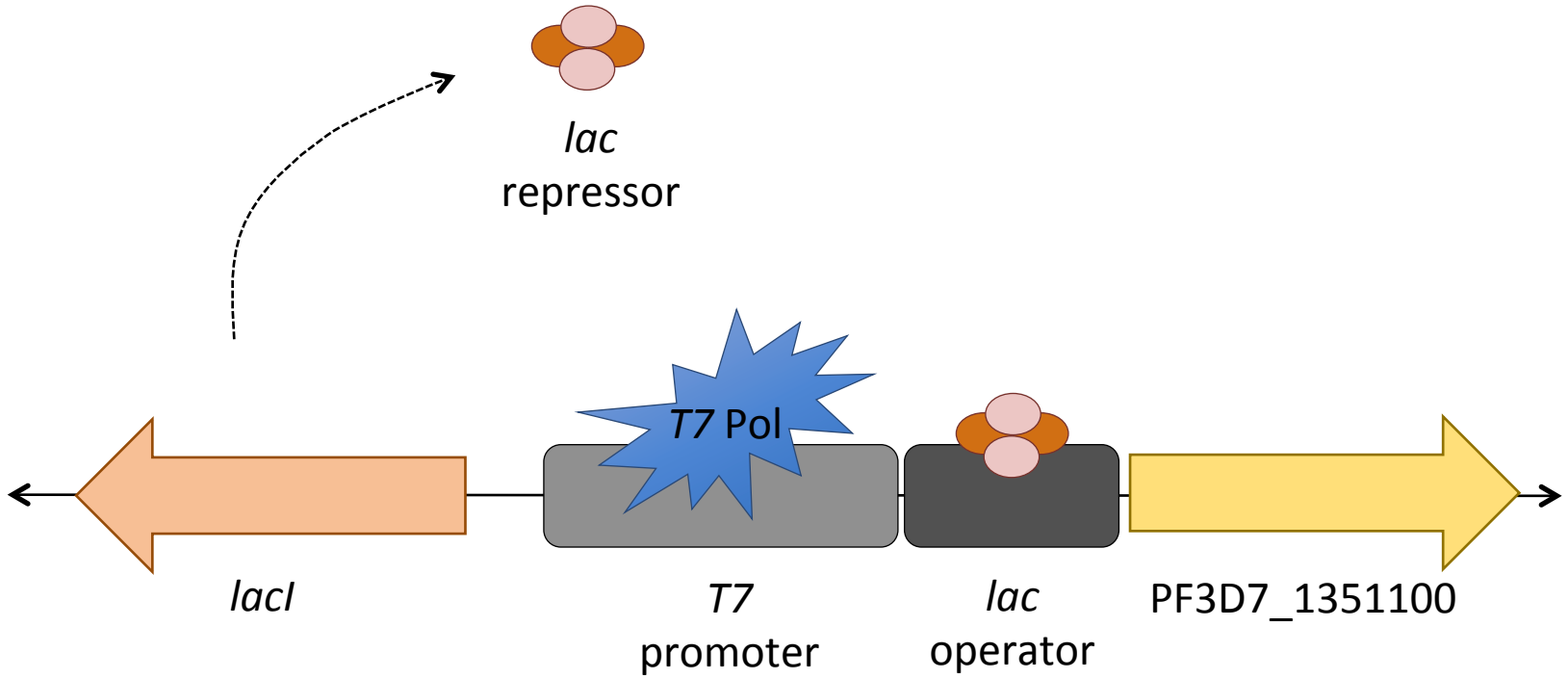


Let’s review protein expression…

- Which polymerase transcribes the protein of interest?
- How is transcription controlled?

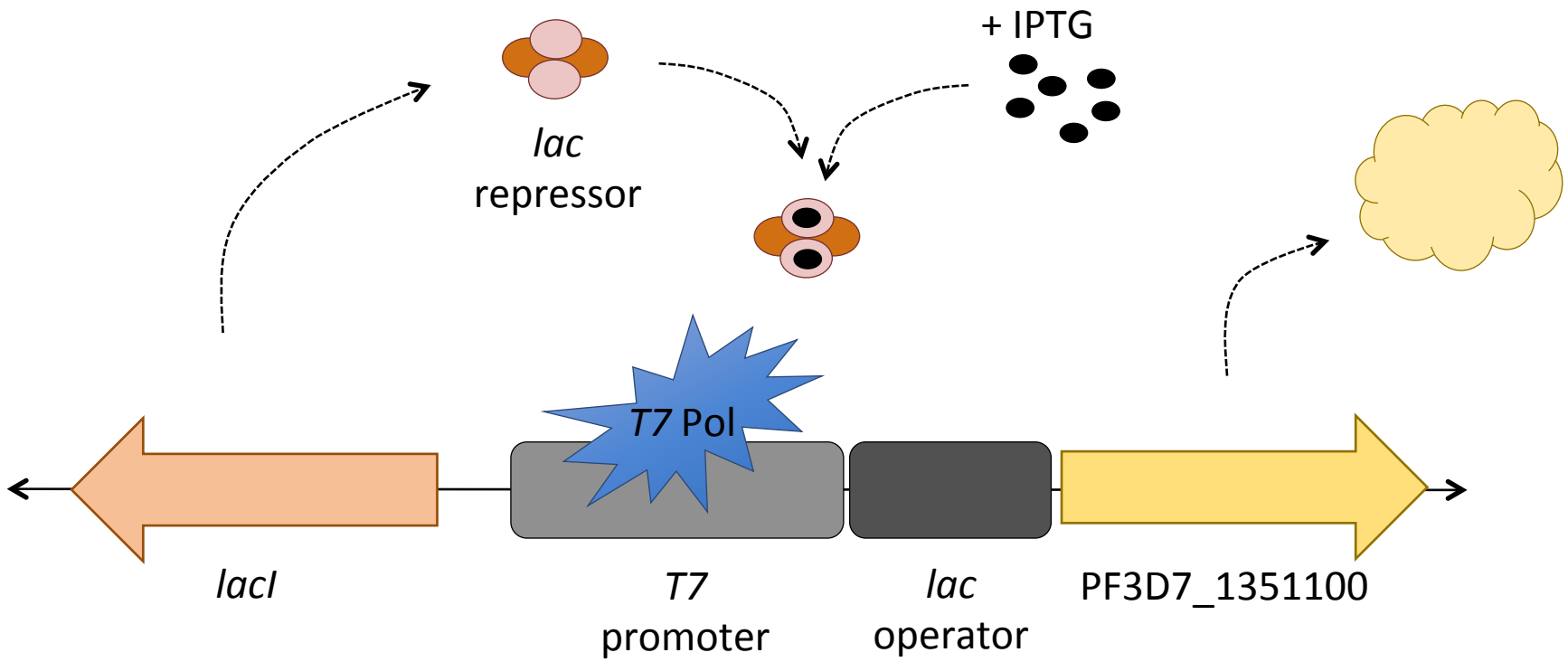


LacI repressor blocks T7 polymerase



pET-28b(+)_PF3D7_1351100

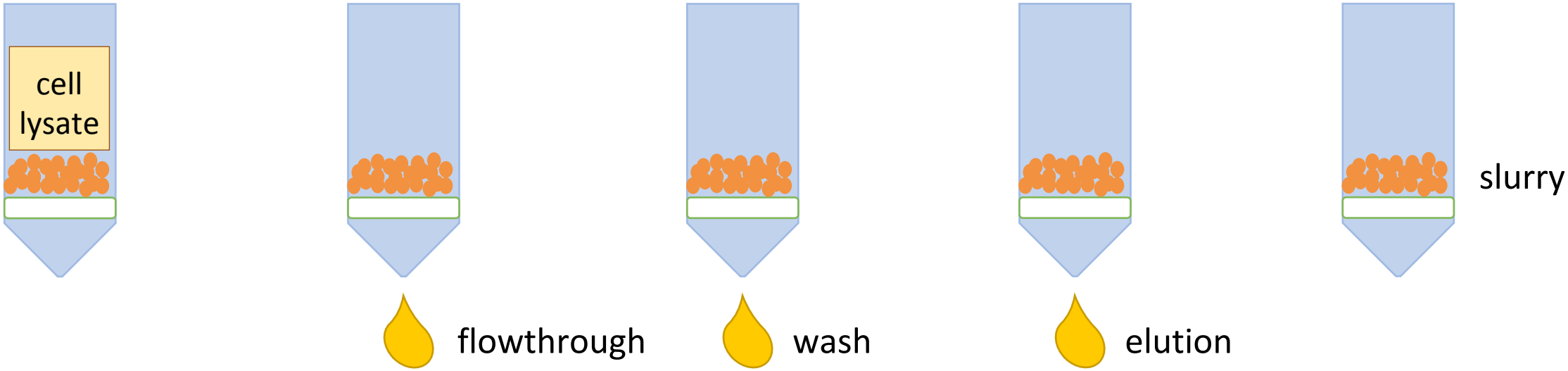
IPTG ‘induces’ protein expression



pET-28b(+)_PF3D7_1351100

Let’s review protein purification…

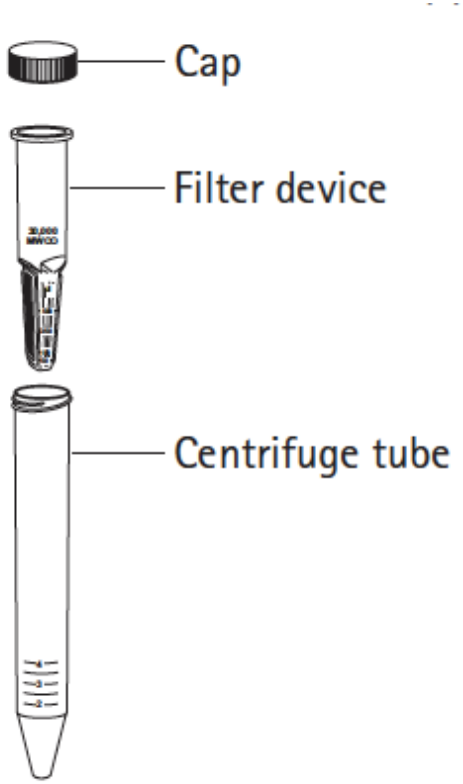
At each step: 1. Purpose 2. On the column? 3. In the liquid?



1.
2.
3.

Protein is concentrated after purification

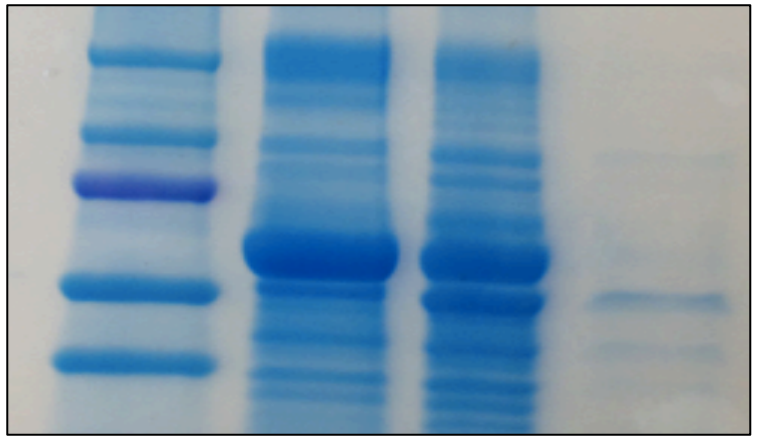
- Filter device sits within centrifuge tube… add protein to filter device for centrifugation
- Filter device has MW cutoff of 3 kDa … protein is retained in the filter device during centrifugation
  - PF3D7_1351100 = 17.5 kDa
  - 6x His tag = 2.5 kDa



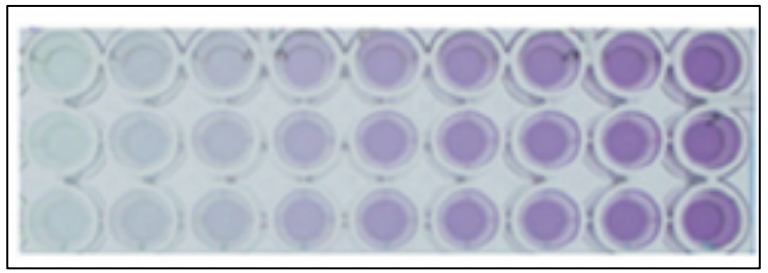
- How does this concentrate the protein?

How will you assess purity and concentration?

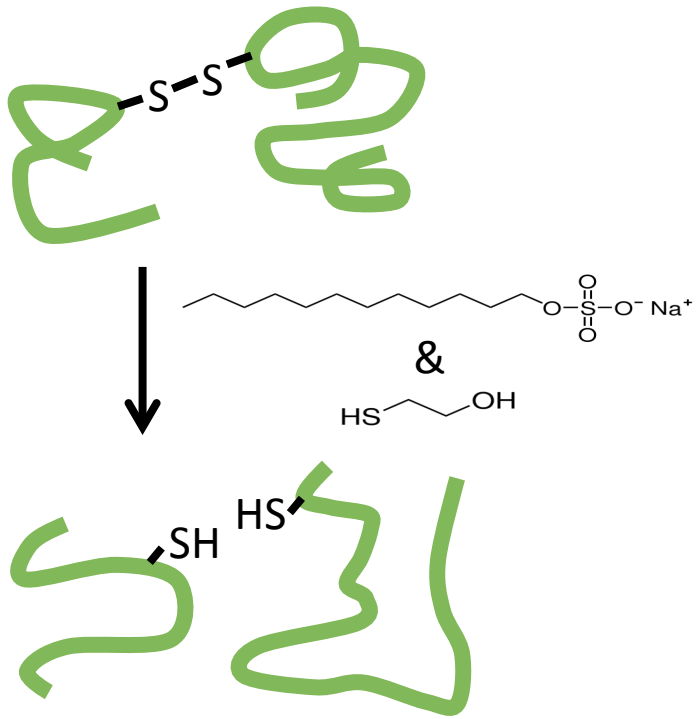
- Check purity using SDS-PAGE
  - Identifies presence of protein during purification procedure
  - Visual detection of other proteins in sample



- Measure concentration using BCA assay
  - Colorimetric assay
  - Calculate concentration from standard curve



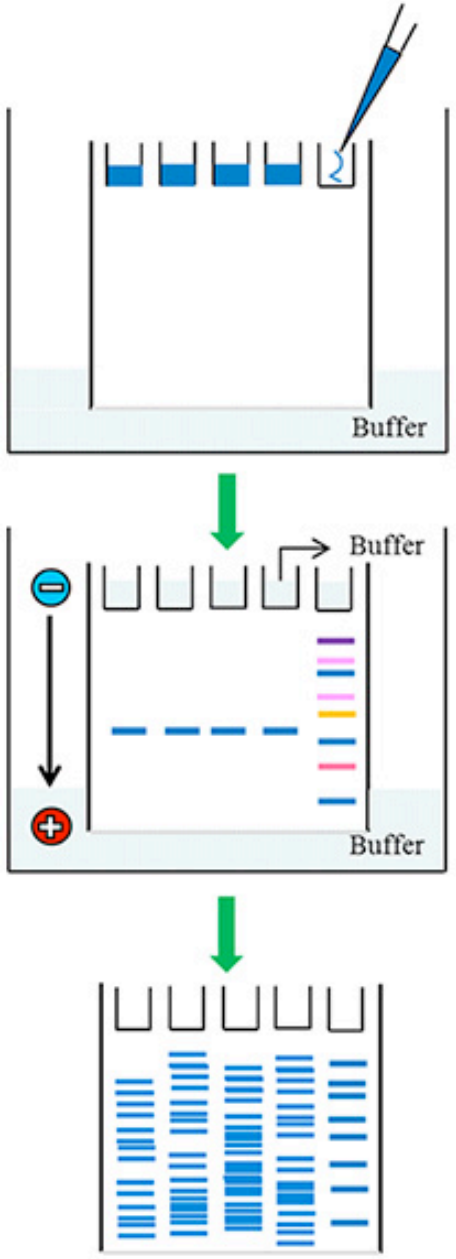
Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



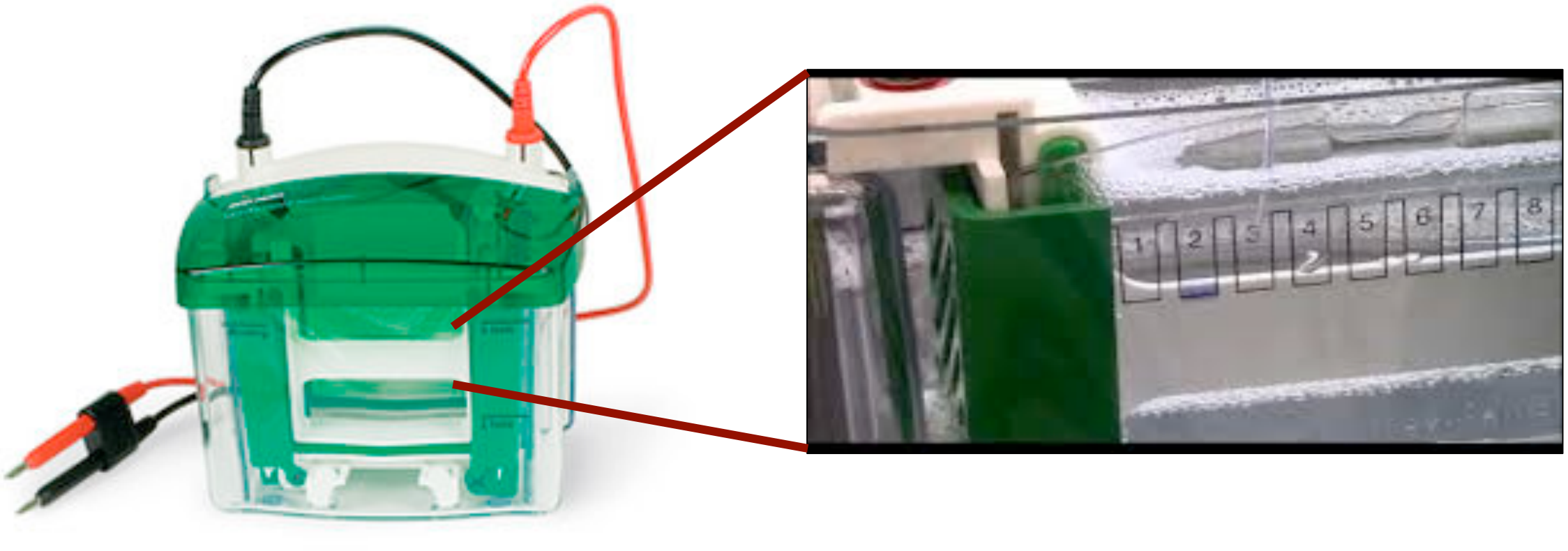
- Laemmli sample buffer / loading dye:
  - SDS
  - β-mercaptoethanol (BME)
  - bromophenol blue
  - glycerol
- Boiling:

How are proteins separated?

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



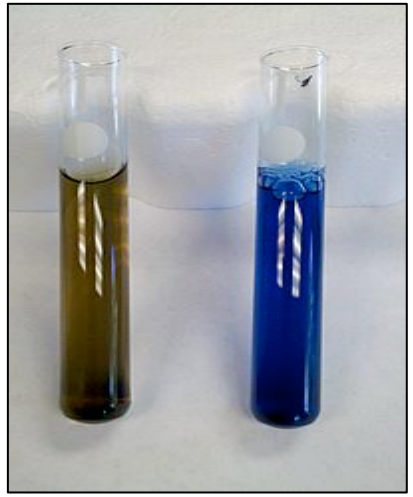
Demonstration of SDS-PAGE

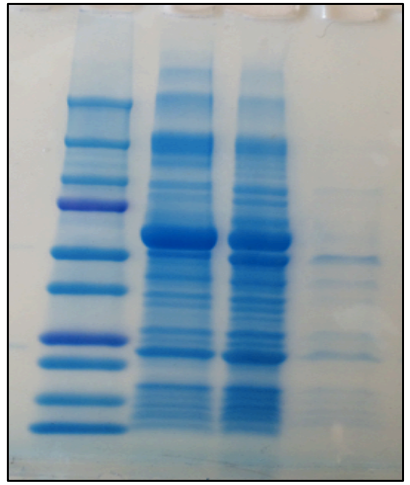


How are proteins visualized?

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)

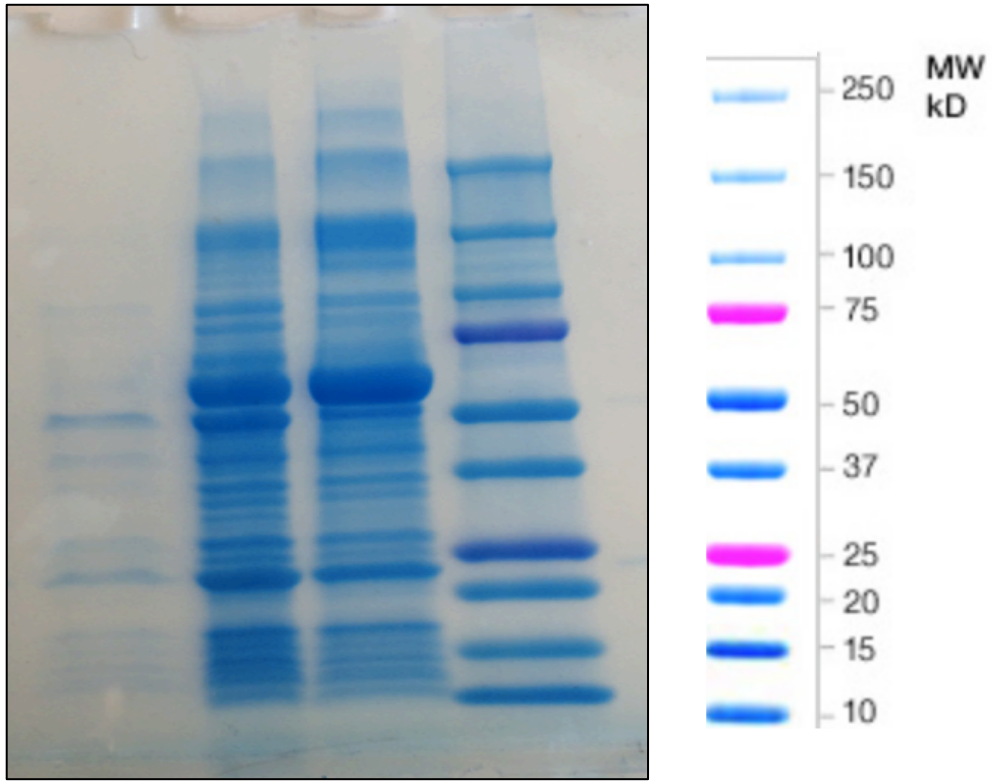




What are the expected results of SDS-PAGE?

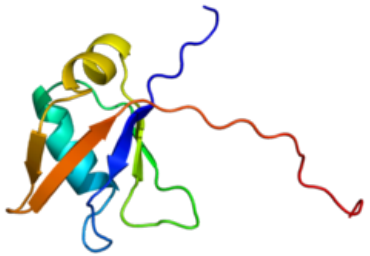
Each lane of the gel should be explained in the results

- What bands are expected? Do you see the bands you expected?
- Do you see any unexpected bands?
- What do the bands tell you about the purity of your protein?
- What does might this tell you about the protein concentration calculated in the next step?



Concentration: Bicinchoninic acid (BCA) protein assay

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



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

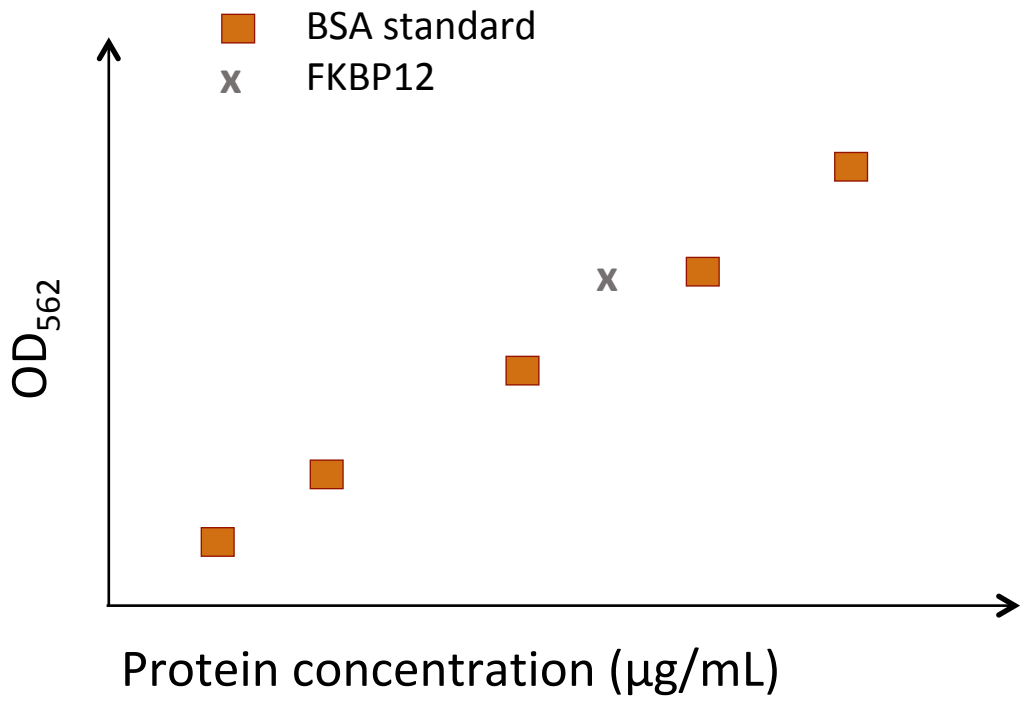
Step 2: BCA complexes with cuprous cation

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

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

Standard curve generated using serial dilutions of bovine serum albumin (BSA)

- Equation of the line used to calculate protein concentration
- What does the R^2 value tell you about the standard curve? What does this tell you about the calculated concentration?



For today…

- Complete protein purification and concentration exercises
- Important note: the confirmation digest and protein assessments will be included as part of the data in your Research article

For M2D4…

- Draft methods for M2D1-M2D3
- Send your availability for Oct 20 and Oct 22!!

To get you started on your methods…

Confirmation digest of pET-28b(+)_PF3D7_1351100

[Include topic / introductory sentence] To confirm that PF3D7_1351100 was cloned into pET-28b(+) expression vector (give credit to Dr. Khan Osman!), a digest was completed. Restriction enzymes XYZ and ABC were used to digest X ng of pET-28b(+)_PF3D7_1351100 in single digests (only one enzyme added) and in a double digest (both enzymes added) using Y U / uL of each enzyme and 1X CutSmart buffer (NEB). Digests were incubated at 37C for Z hrs. …