Notes for Data Summary:

- If you would like to do revisions on your Data Summary
  - These are not required

- Revisions have the potential to bring a grade up 1 & 1/3 letter grade (i.e. B- to A)

- We run revisions in the same way as scientific journals
    - Write a cover letter!
    - We ask that you go one step further and show your changes with different colored font

> **Data summary revision is due by Saturday, October 23rd at 10 pm** [edit]
>
> **For your final report, all changes need to be in a different colored font so the improvements you made are clear**. You should also include a cover letter with your final draft that explains how you addressed the concerns raised (*e.g.* "paragraph x was completely rewritten to better explain….” or “Results for the agarose gel analysis were clarified by ….").

Notes for Journal Club:

Journal club meets in 56-614 on T/R

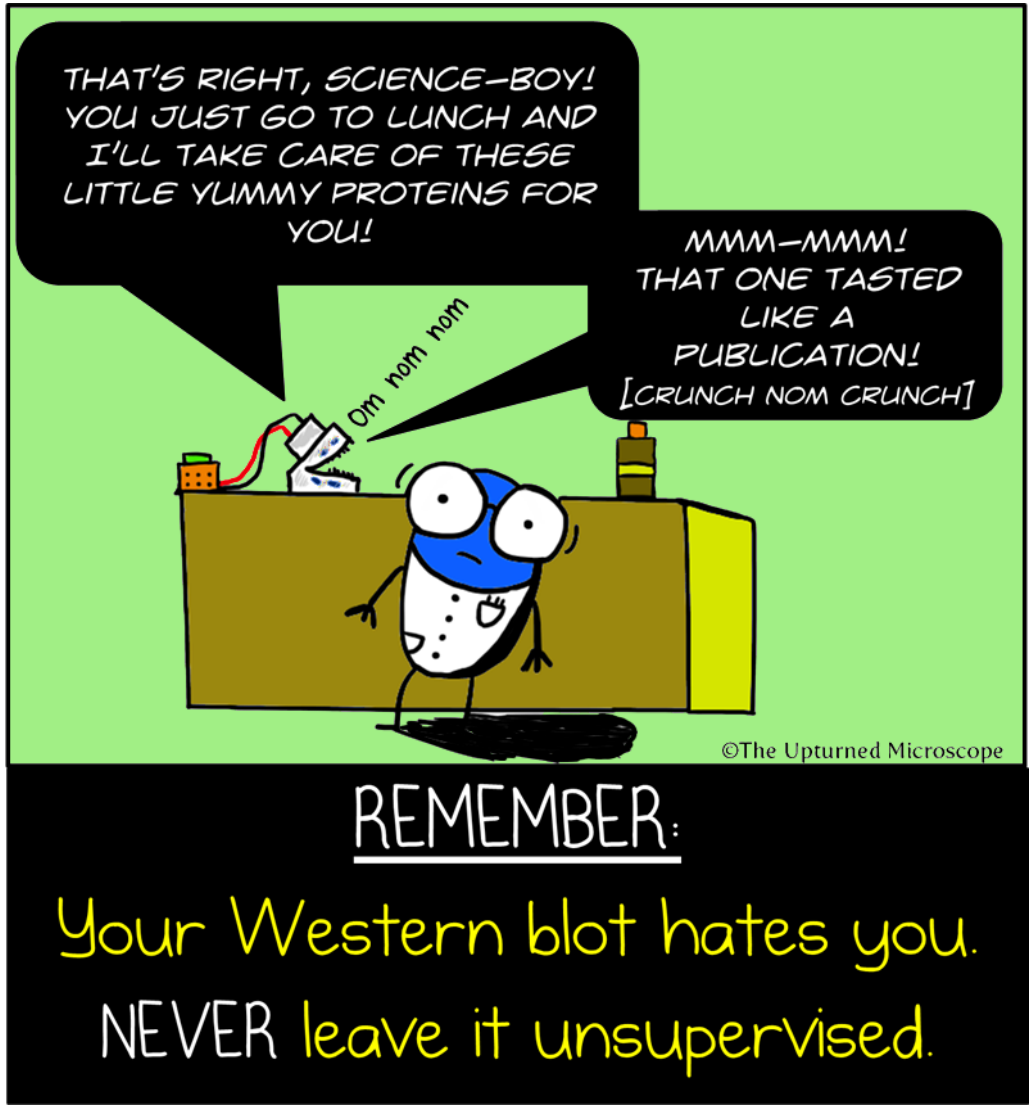
- If you want to discuss your article, please email in advance (to make sure I've read it)
- Your slides must be uploaded to Stellar 1hr prior to the class day in which you are presenting
  - The earliest slide upload gets first choice of a presentation slot on that day, and so on…
- We provide a Trader Joes smorgasbord to fortify through this experience
  - Let me know about food requests and any dietary restrictions
- No lecture on Tuesday or Thursday of next week

Method of submission [edit]

Please submit your completed Journal club slides **1 hr prior to your scheduled laboratory session time** to Stellar, with filename **Name_LabSection_JC.pptx** (for example, ImaStudent_TR_JC.pptx).

M2D5: Assess purity and concentration of purified protein

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



Module 2 Roadmap

Determine putative PF3D7_20109-F21 binders via high throughput screening (SMM)

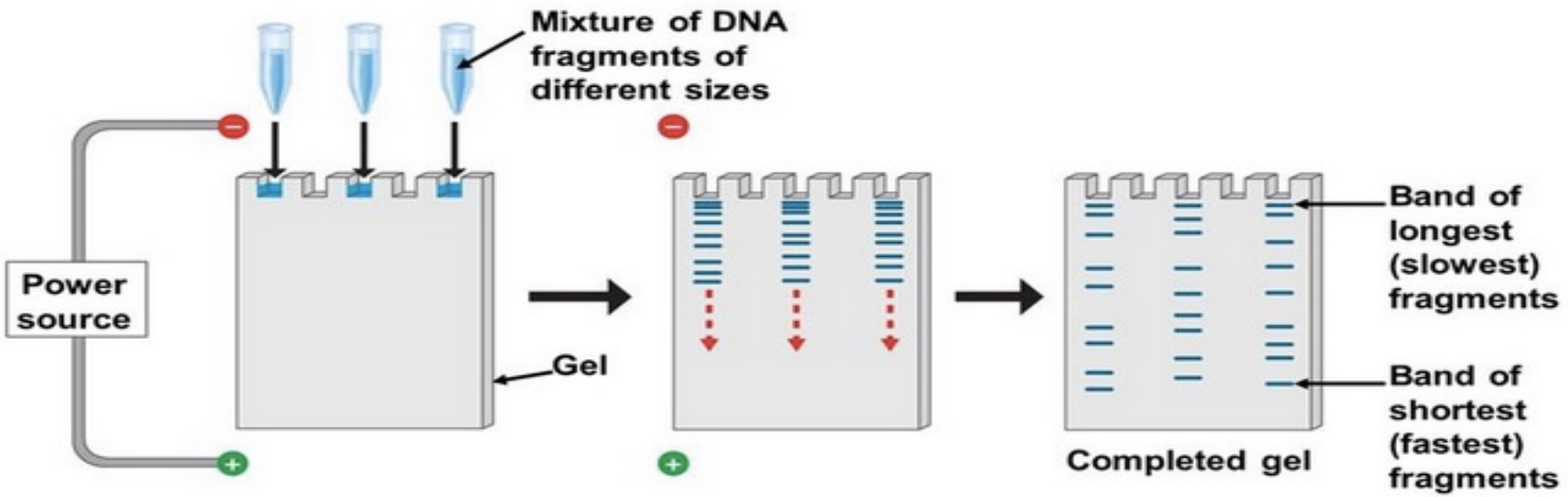
Create plasmid of PF3D7_20109-F21 to use in validation assays

Express PF3D7_20109-F21 (from plasmid) in bacteria and purify protein

Assess purity and concentration of purified protein

Use purified protein to validate binding of small molecules identified in SMM

DNA electrophoresis review



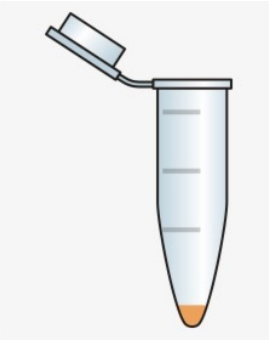
How do you visualize the migration through the gel?

How do you visualize DNA bands in the gel?

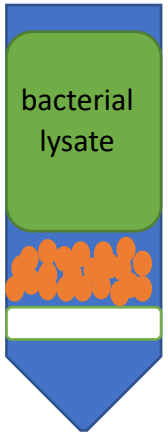
Protein purification review

- Why this step?

Pellet



Lysate

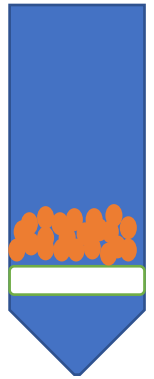


Flowthrough

Wash

Elution

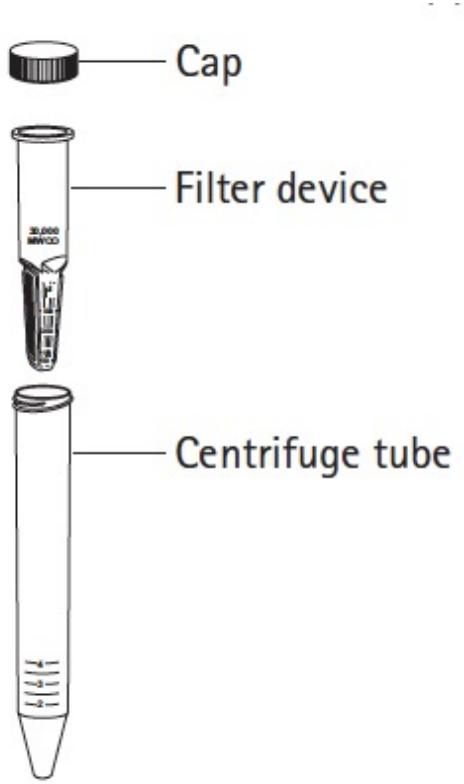
Slurry



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

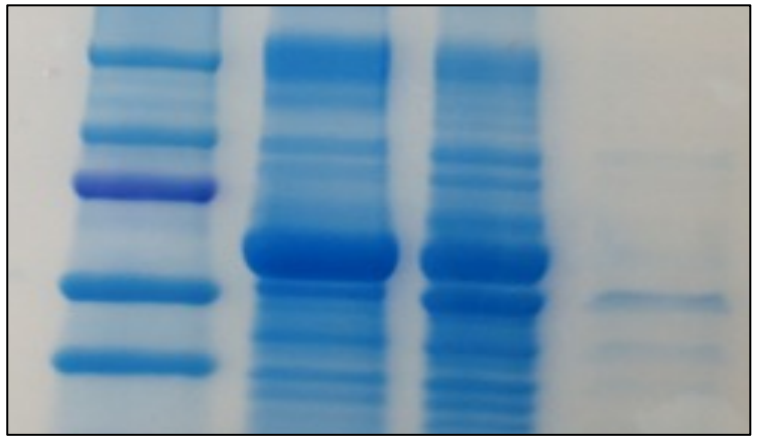
Concentrate protein before testing

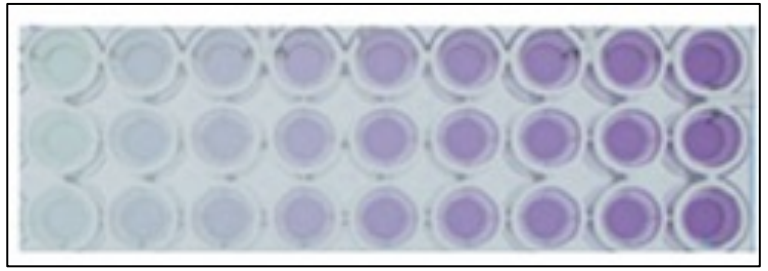
- Filter device sits within centrifuge tube…add protein to filter device for centrifugation
- Filter device has MW cutoff of 3 kDa …protein is retained in the filter device during centrifugation
  - **PF3D7_1351100 = 23kDa**
  - **Strep-tag = 3kDa**
- How does this concentrate the protein?
- How does this remove excess biotin?



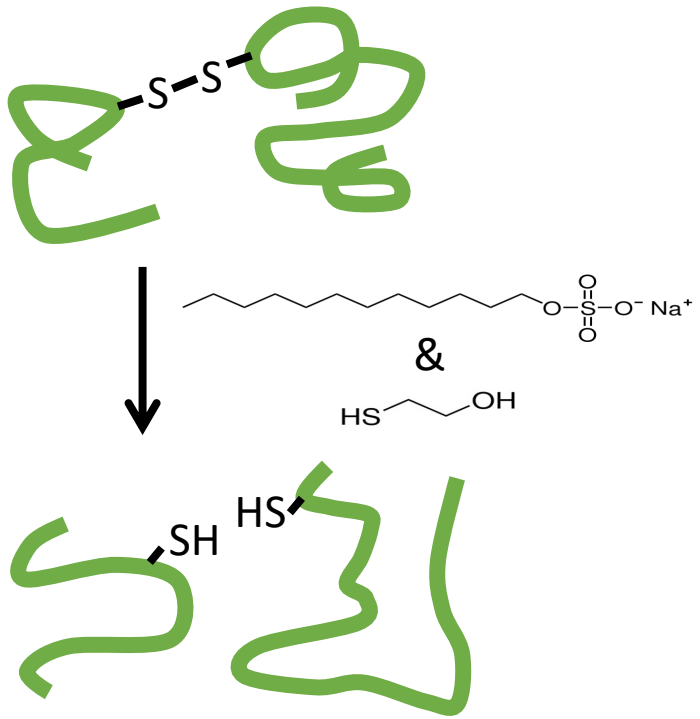
How will you assess purity and concentration?

- 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





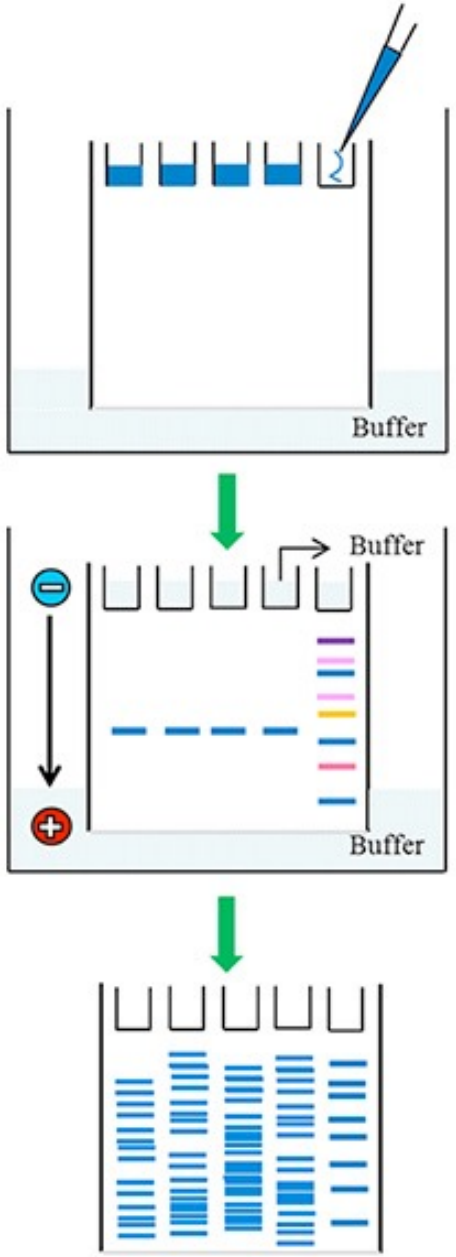
Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



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

How are proteins separated?

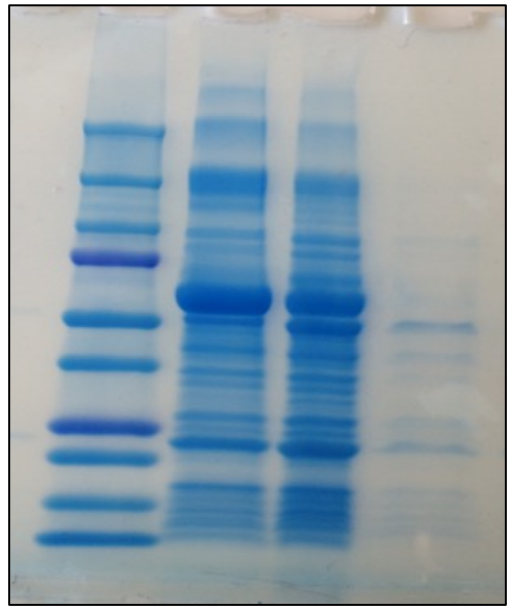
- Laemmli buffer and boiling results in _____ and _____ charged proteins
- SDS-PAGE separates proteins by _____
- Electrophoresis completed in TGS buffer
  - Tris-HCl
  - SDS
  - Glycine



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

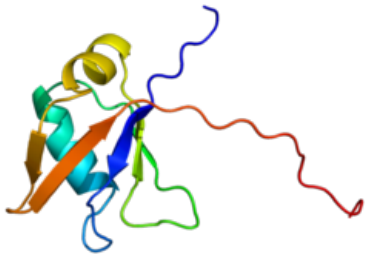
Consider the order of your samples:

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+}

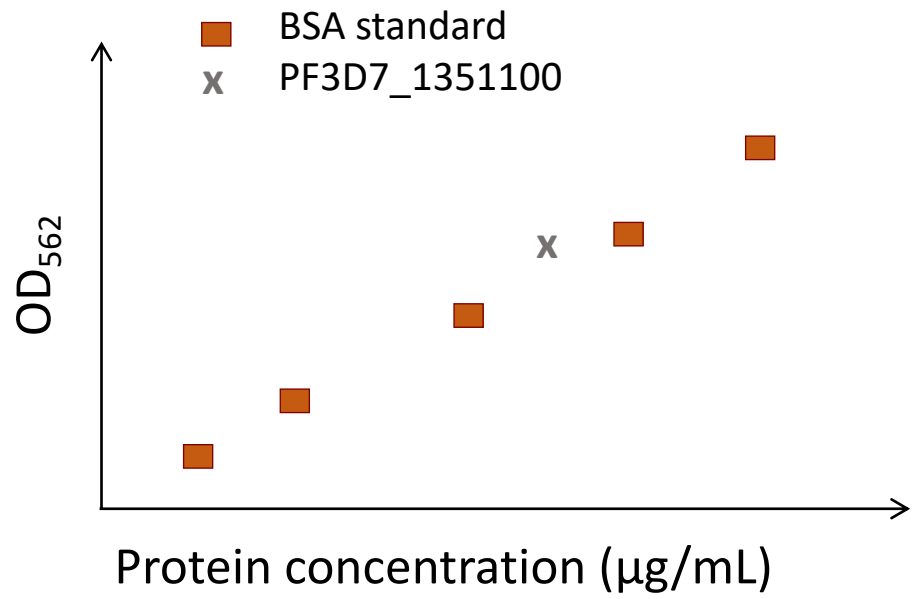
Step 2: BCA complexes with cuprous cation

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 M2D5…

- Draft methods for M2D2-M2D4
  - For your research article
  - Individual assignment

Methods gift

Confirmation digest of pET-28b(+)_PF3D7_20109-F21

Topic sentence! (Include mention that construct was a gift from Dr. Khan Osman)

To confirm that PF3D7_20109-F21 was cloned into pET-28b(+) expression vector, a digest was completed. Restriction enzymes AbcI and DefIII were used to digest X ng of pET-28b(+)_PF3D7_20109-F21 in single digests (only one enzyme added) and in a double digest (both enzymes added) using Y U / uL of each enzyme and CutSmart buffer (NEB). Digests were incubated at 37C for Z hrs. …