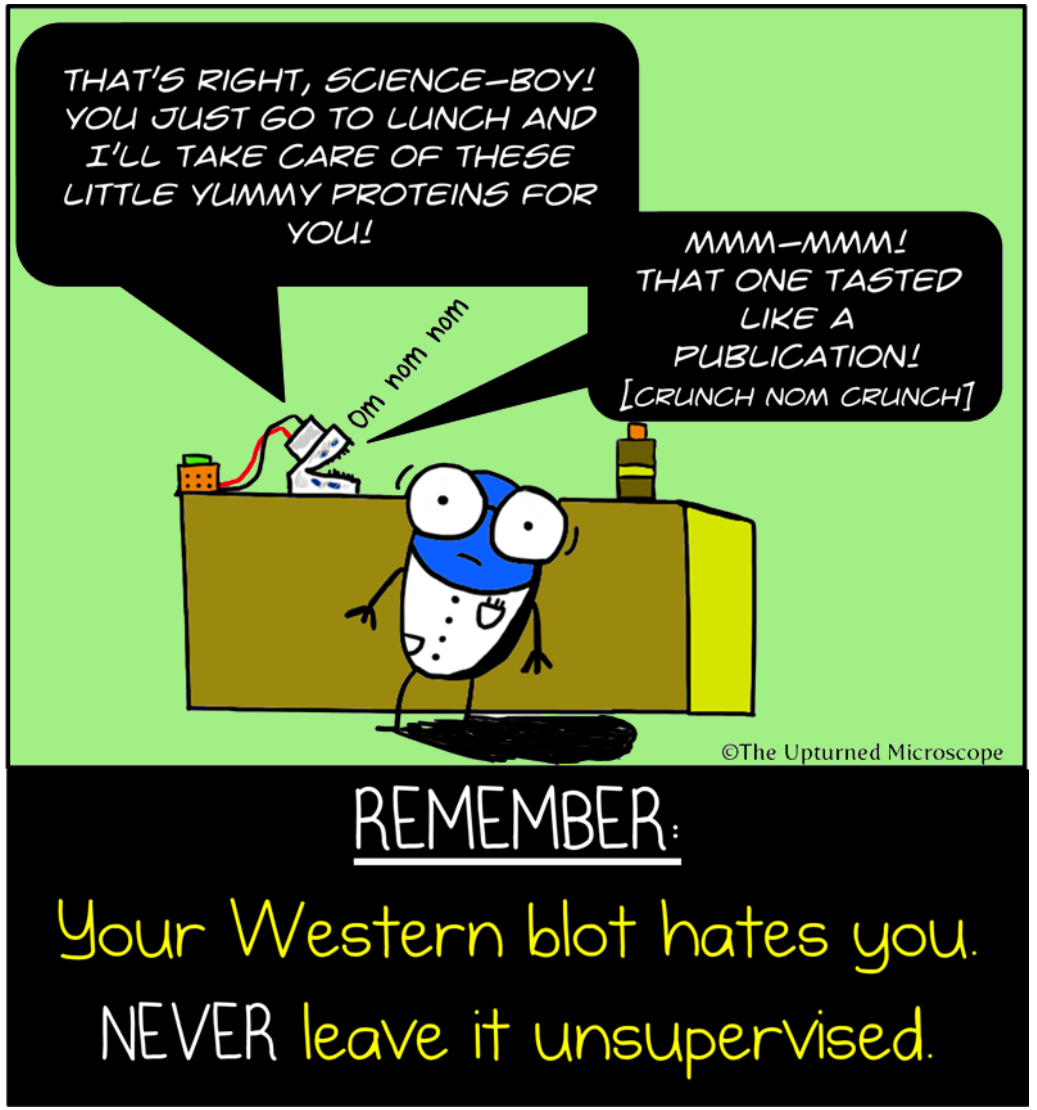
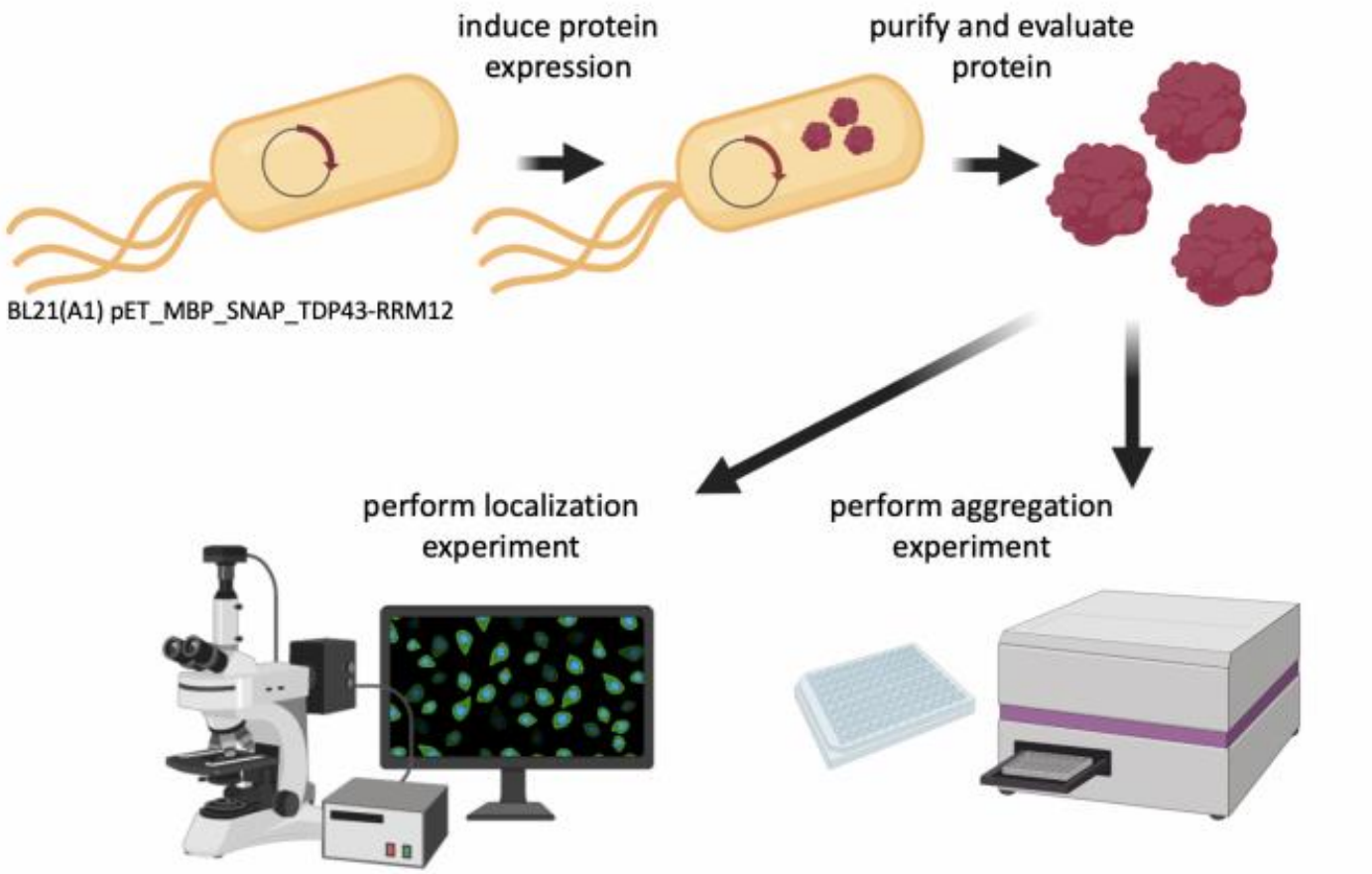
M1D4: Assess purity and concentration of purified protein

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



Overview of Mod 1 experiments

Research goal: Use functional assays to characterize ligands identified as binders to TDP43 from SMM technology

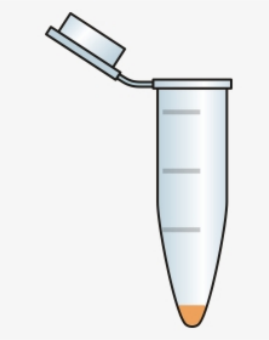


Protein purification review

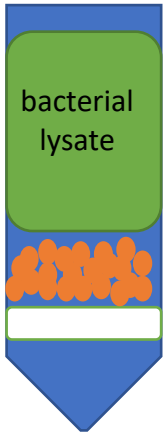
- Why this step?

- What's on the resin? (Ideally)
- What's in the expelled liquid? (Ideally)

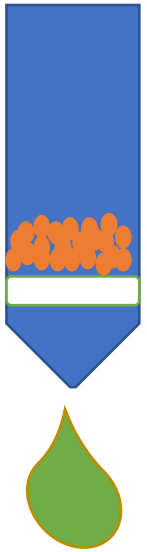
Pellet



Lysate



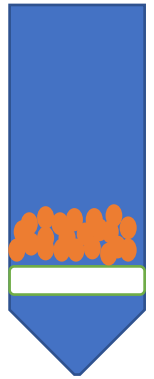
Flowthrough



Wash

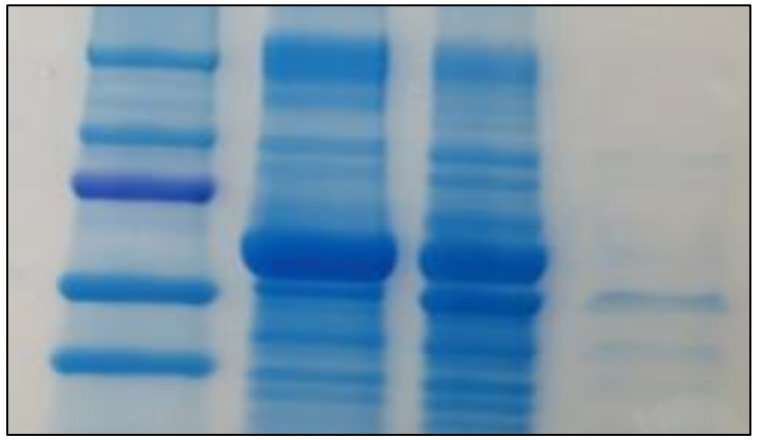
Elution

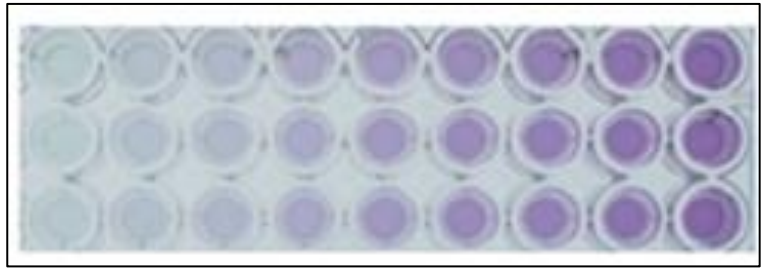
Slurry



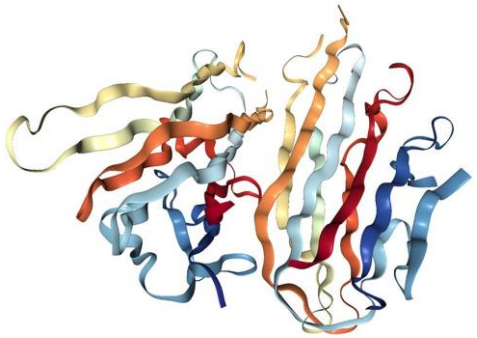
How will you assess purity and concentration?

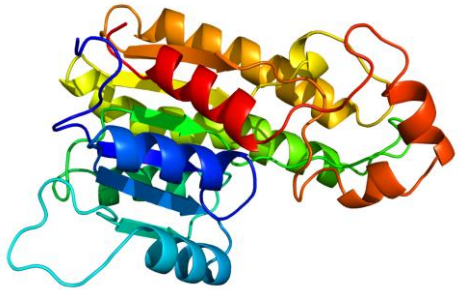
- Check purity using SDS-PAGE
  - Visual detection of all proteins in sample
  - Identifies purity of sample at multiple stages of purification
- Measure concentration using BCA assay
  - Colorimetric assay
  - Calculate concentration from standard curve

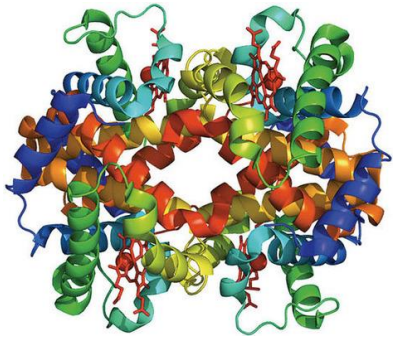




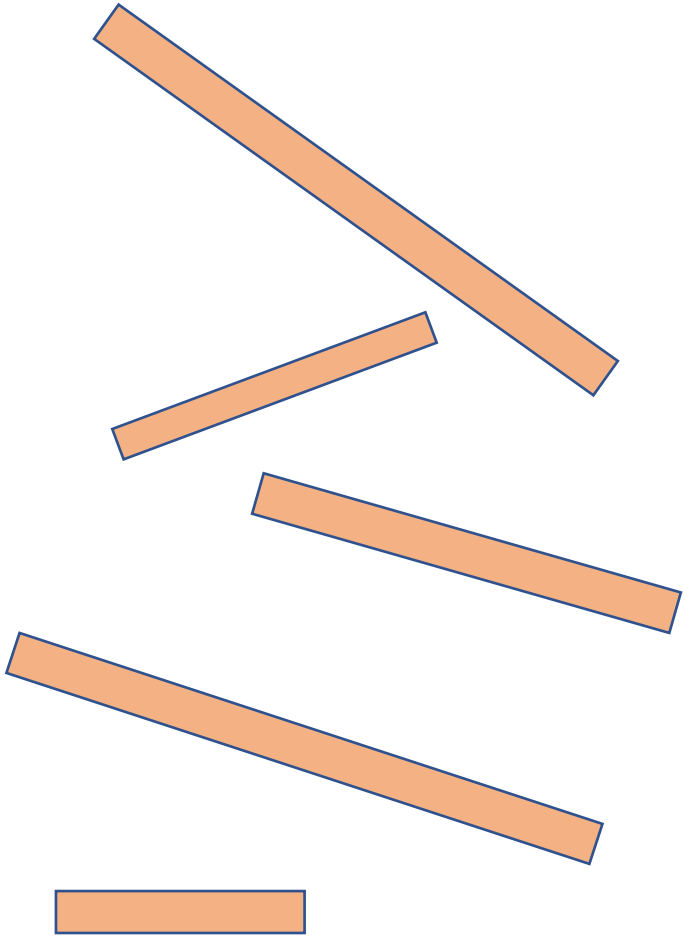
SDS-PAGE is a denaturing gel



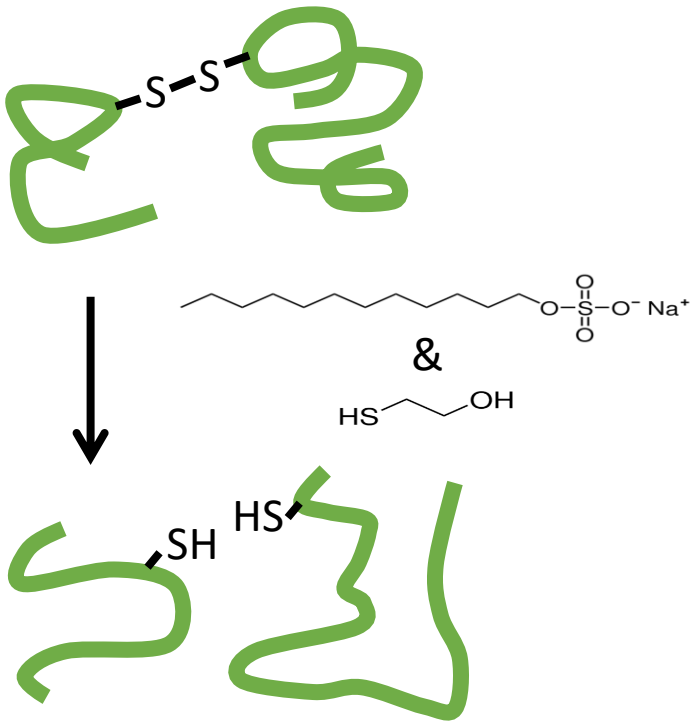




Why run a denaturing gel?



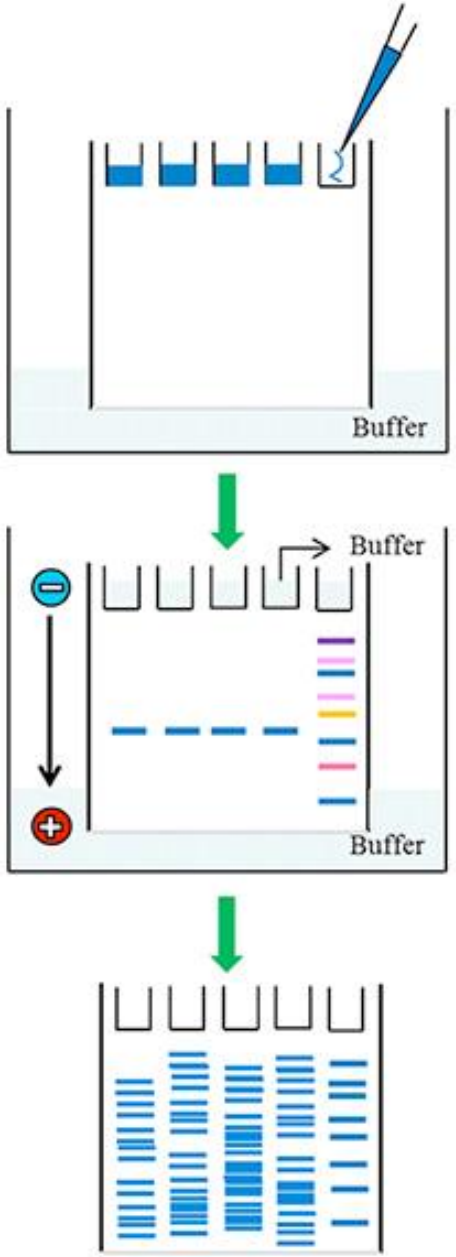
Purity: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)



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

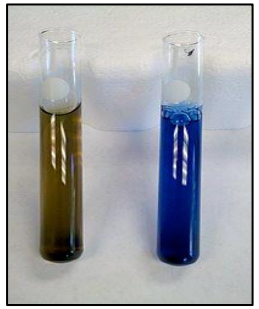
How are proteins separated?

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

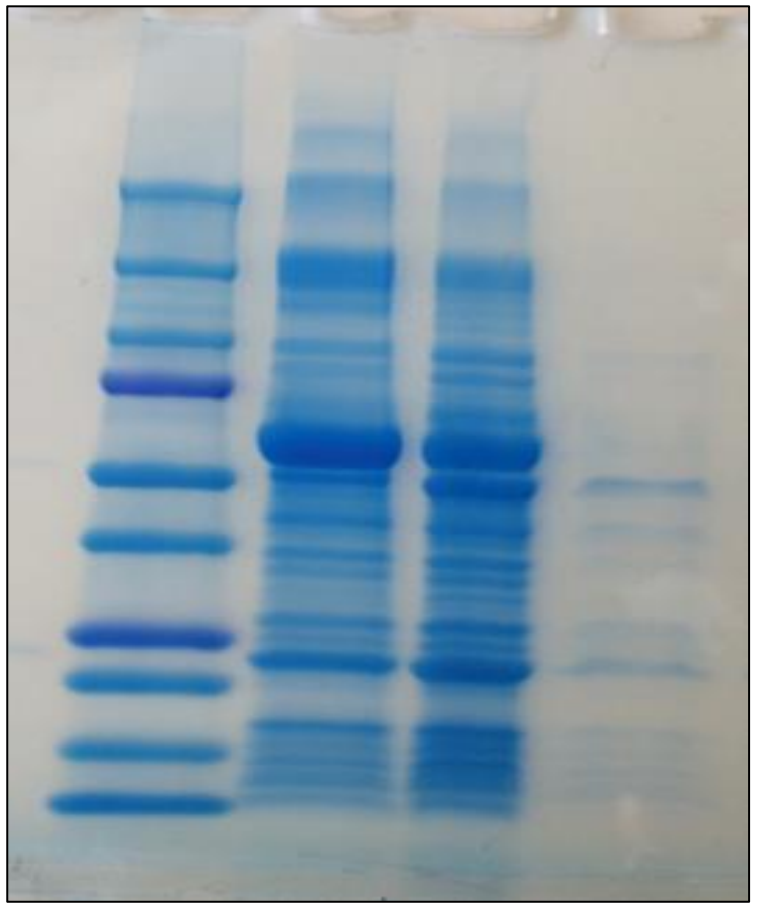


How are proteins visualized?

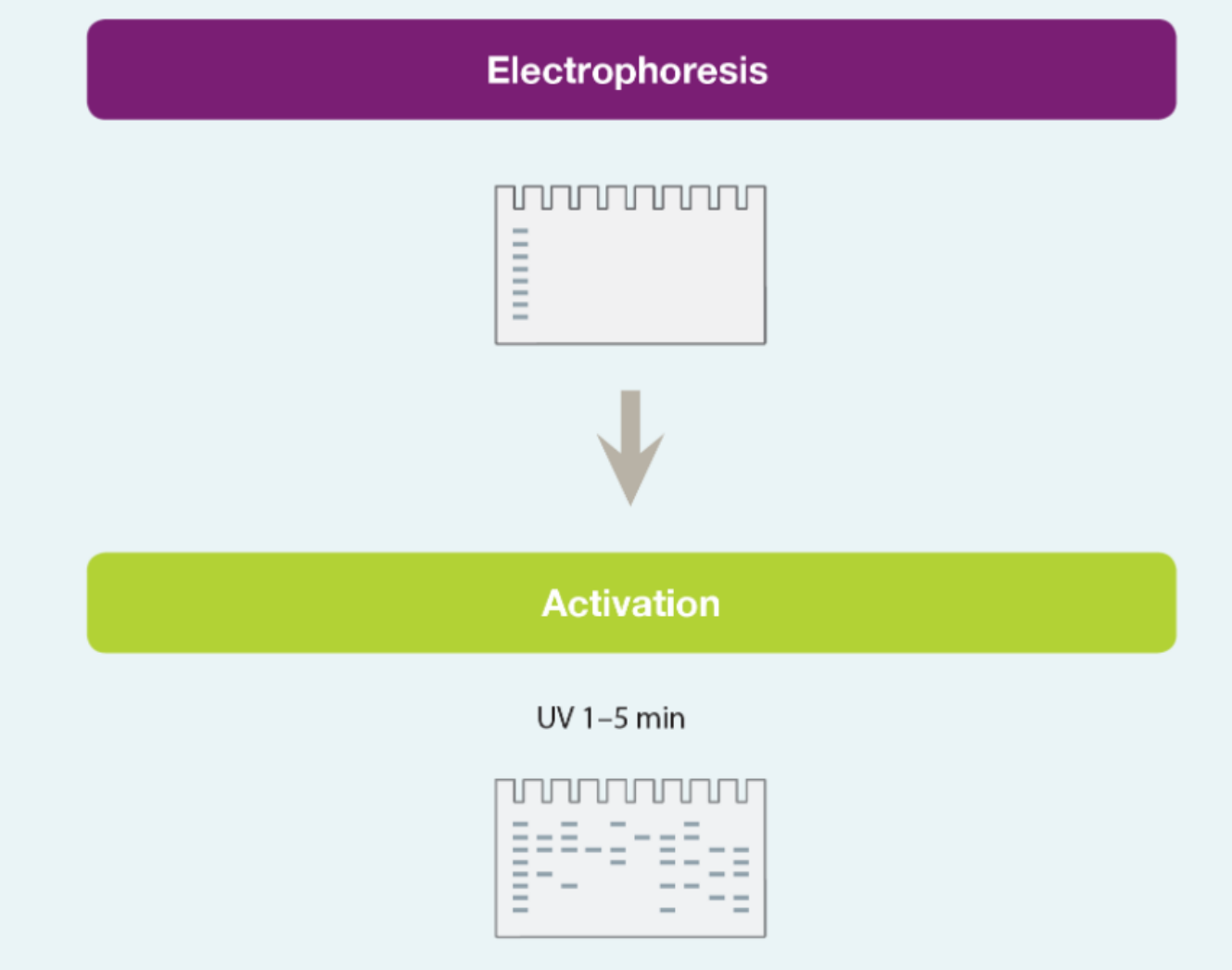
Coomassie brilliant blue G-250 dye used to stain gel after electrophoresis



- 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)



How are proteins visualized? (Today?)



Trihalo compound binds tryptophan and generates a fluorescent signal

Be mindful when assessing SDS-PAGE protein samples

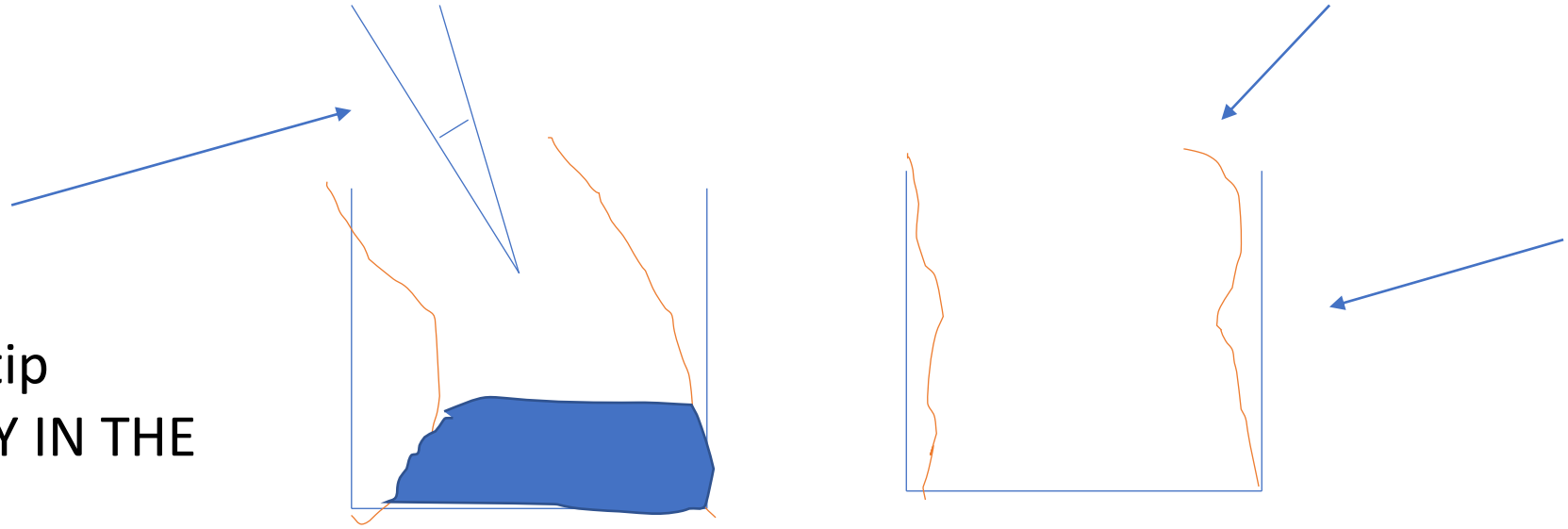
Consider the order of your samples:

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

- Figure will be included in your Data Summary!

Gel loading pro tips – Look!! With your 👁👁

Real Well

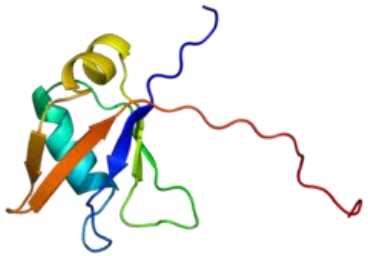


Pipette tip VISUALLY IN THE WELL, BETWEEN PLATES

Lane GUIDE (actual mileage may vary)

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+}

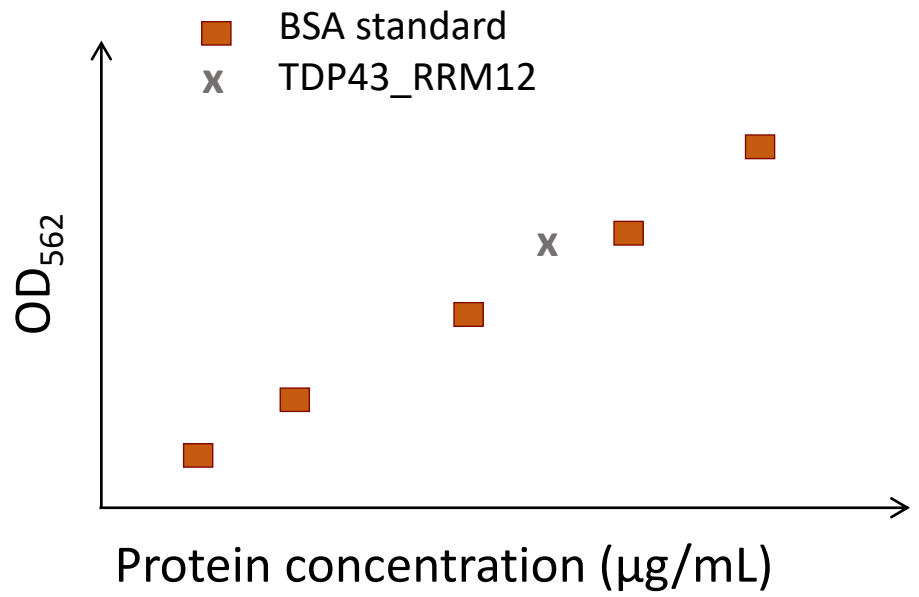
Step 2: BCA complexes with cuprous cation

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

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

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

- Use fresh tips between tubes
- Mix well between dilutions
- Be mindful of volumes



Pro Tip: Stock BSA only used for Tube A-C

Tube	Volume of diluent (µL)	Volume of BSA (µL)	Final concentration of BSA (µg/mL)
A	0	300 of stock	2000
B	125	375 of stock	1500
C	325	325 of stock	1000
D	175	175 of B	750
E	325	325 of C	500
F	325	325 of E	250
G	325	325 of F	125
H	400	100 of G	25
I	400	0	0 = blank

Pro tip on level of detail for Methods

Beginner	Intermediate (Methods are here!)	Professional
1) Combine 7 cups of loosely packed flour, 1 tbsp of yeast, 1 tsp of salt in a large mixing bowl and whisk to combine 2) Add 3 cups of warm, but not too hot water. Comfortably warm is good. Too hot and you will kill your yeast. 3) Using a non-stick spatula, begin to mix. When the dough gets too tough to mix, transfer the dough to a floured surface and knead for 15 minutes 4) To knead dough, push with the palm of your hand	1) Combine 7 cups of flour, 1 tbsp yeast, 1 tsp of salt and mix 2) Add 3 cups of warm water and mix. 3) Knead by hand until a seam no longer forms and the dough springs back slowly when pressed 4) Let proof for 1 hour 5) Degass the dough and roll out into a 9x13 rectangle. Roll into a baguette and cut in half 6) Proof for 1 hour 7) Preheat oven to 450 F	1) Make a French bread 

For today…

- Complete the purity and concentration assessments
  - It’s good to divide the work load here!
  - Start by deciding who will be in charge of the two different techniques today

For M1D5…

- Create a data figure of your purification results
  - must include SDS-PAGE gel
- Outline your Research Talk
  - See Assignments page for details and Homework page for checklists
  - Focus is aggregation experiment, just put a placeholder for actual results

Data figure example

- Image should not be the entire page
  - Only needs to be large enough to be clear / visible
- Title should be conclusive
  - Don't include what you did, rather state what you found (take home message)
- Caption should not detail the methods or interpret the data
  - Define abbreviations, symbols, etc.
  - Info needed to "read" figure

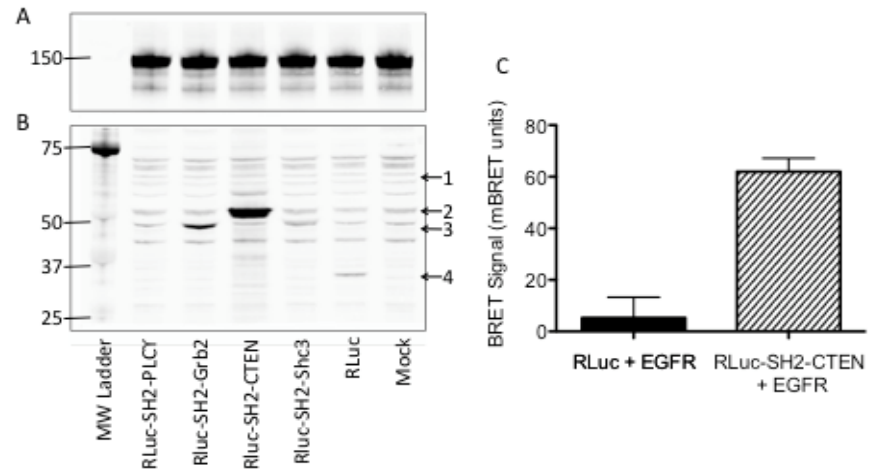


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Shc3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

Notes on the Research Talk

- Individual assignment
- Three (3) minute video of you talking directly into the camera
- No visual aids allowed
  - Introduce yourself and your project
  - Highlight key results with quantitative information
  - Place your work in the scope of the larger field
- No need to state you are doing a class project or anything about 20.109

- Homework = outline
  - Introduction of your project
  - Key results from your research (including a statement as to the method(s) used to generate data)
  - Take-home message