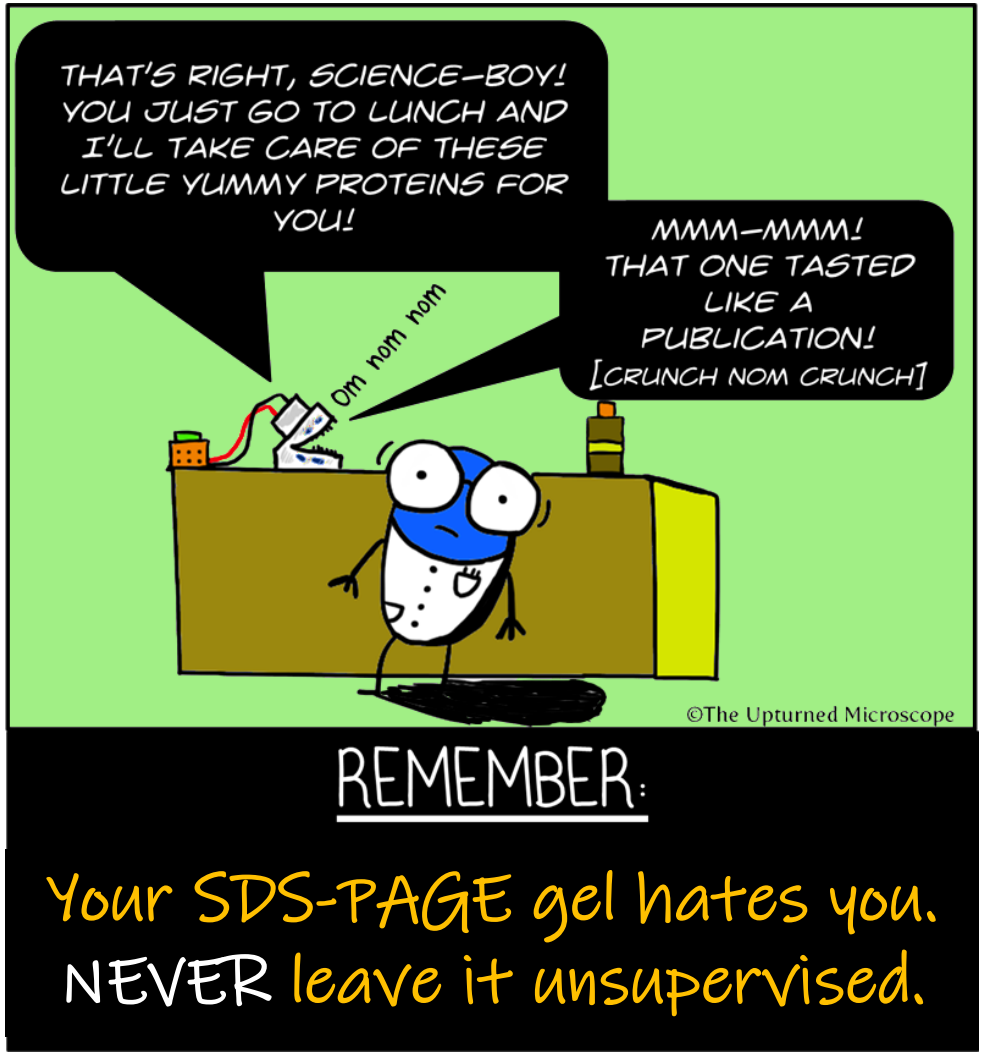
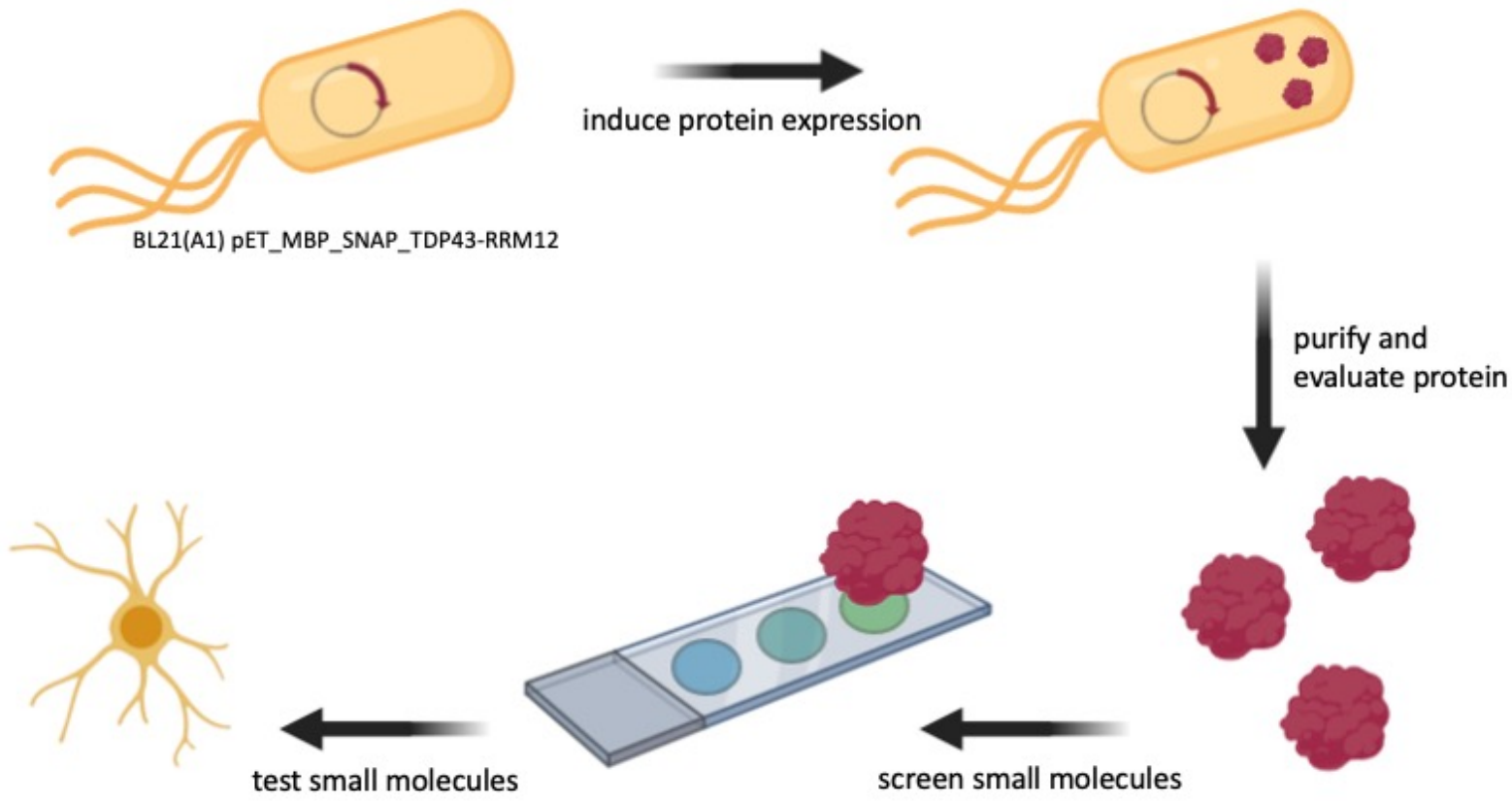
M2D2: Assess purity and concentration of purified protein

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



Overview of M2

Research goal: Identify and characterize small molecule binders to a protein drug target.



Protein induction review

① aggregation potential - specific for TDP43

② stressor for bacteria

- What were the two chemicals used to induce TDP43_RRM12 expression?

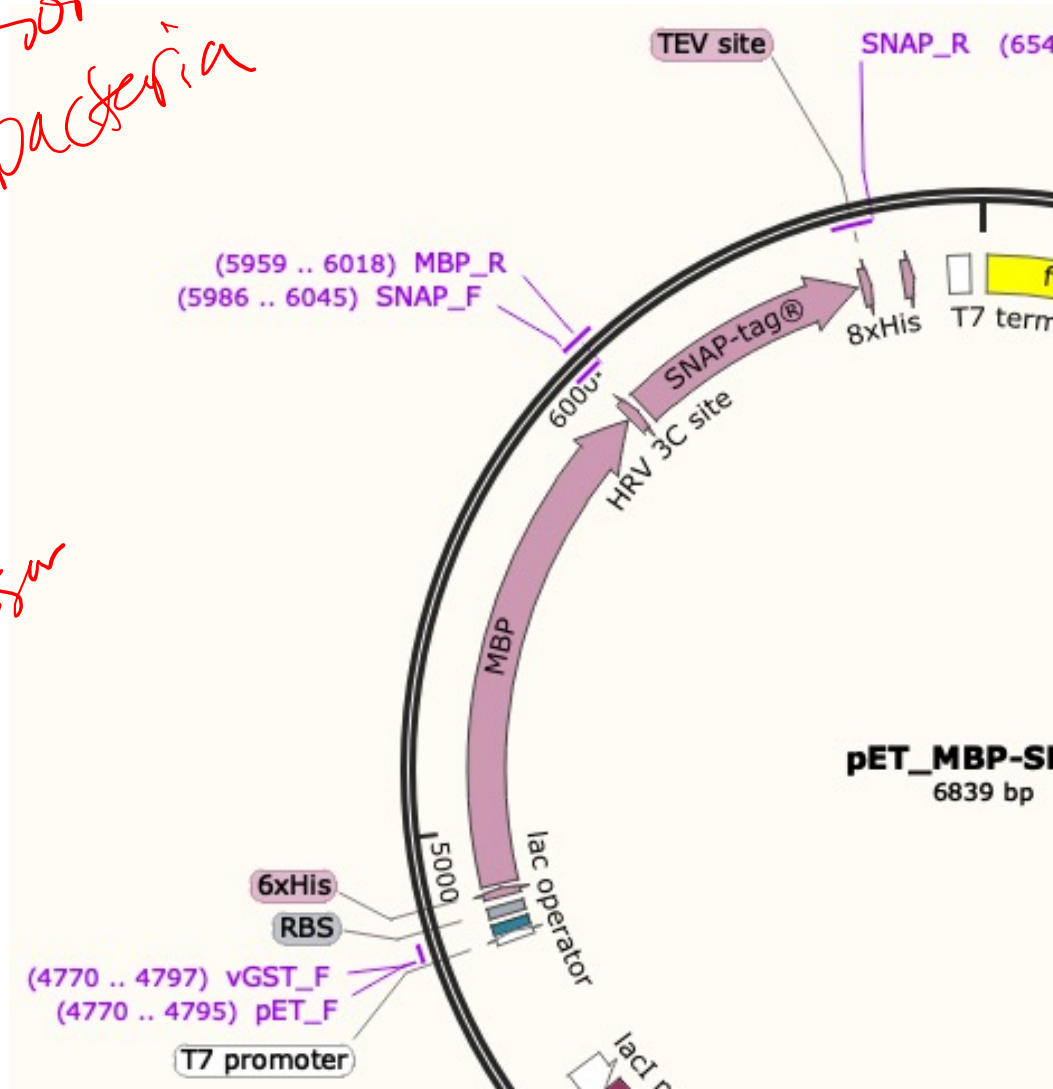
Arabinose

IPTG

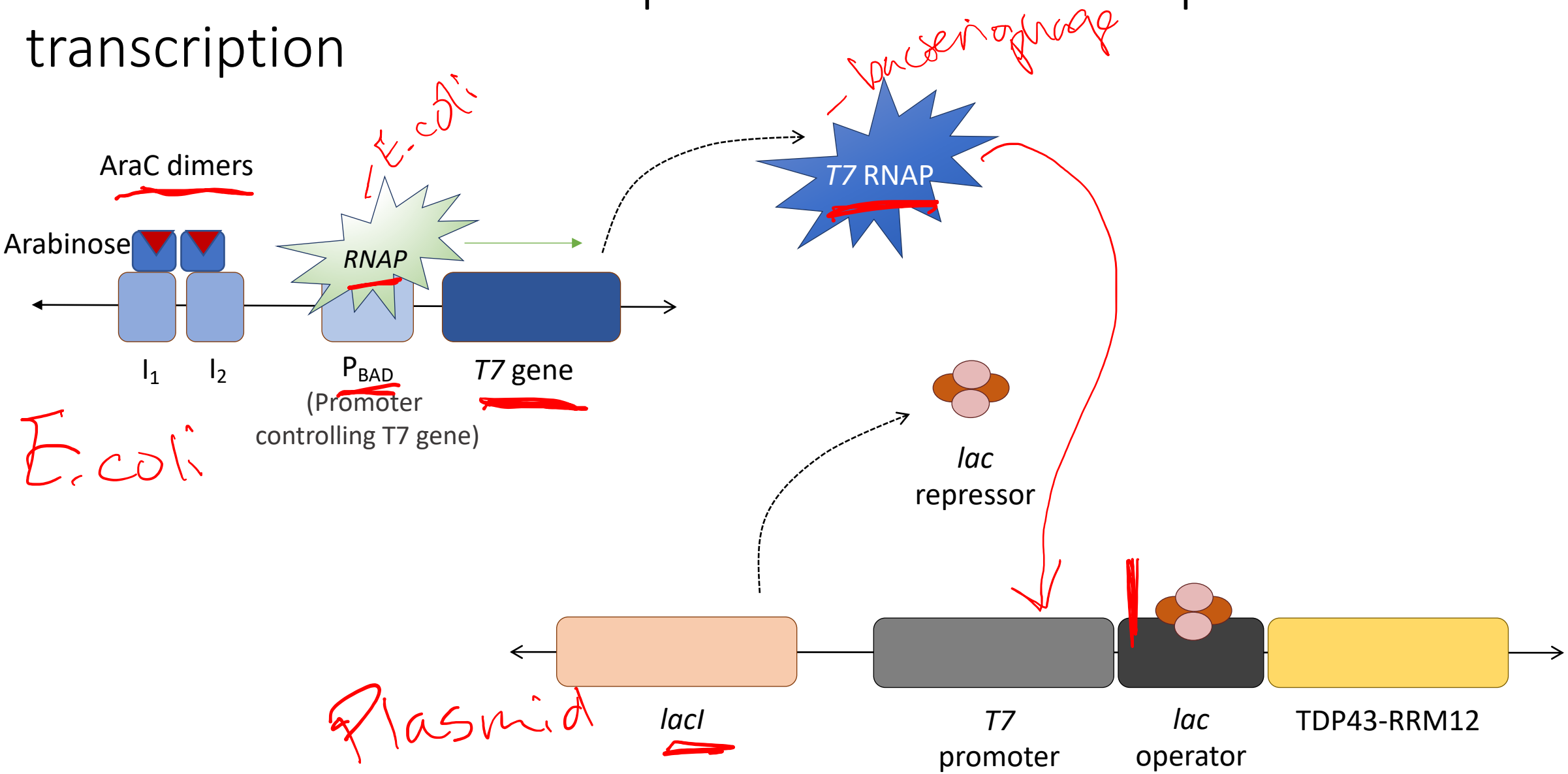
- What do they allow to be expressed/how?

IPTG = lactose analog - bind lac repressor

Arabinose = T7 RNAP

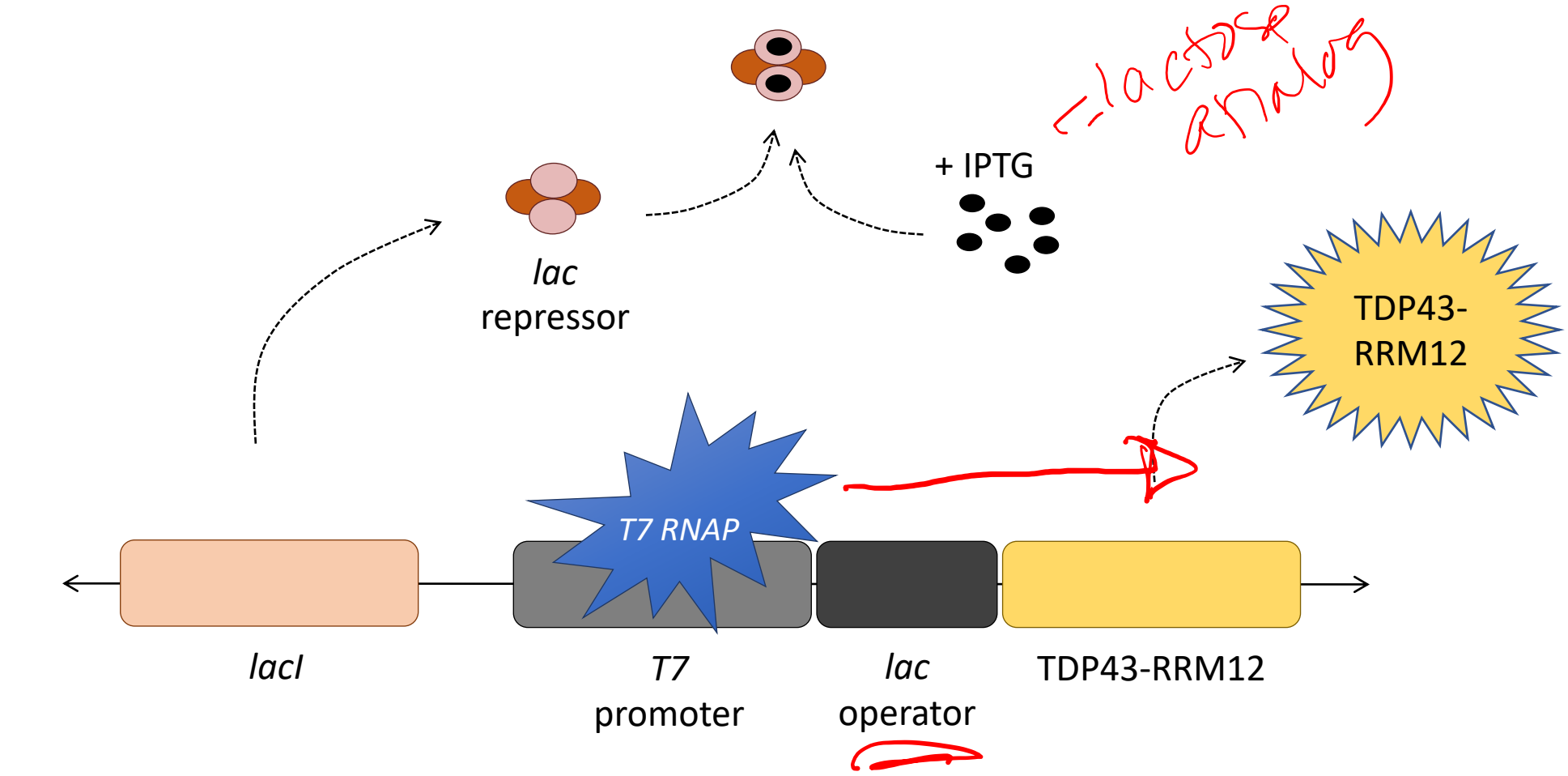


Arabinose controls T7 expression while LacI repressor blocks transcription



pET_MBP_SNAP_TDP43-RRM12

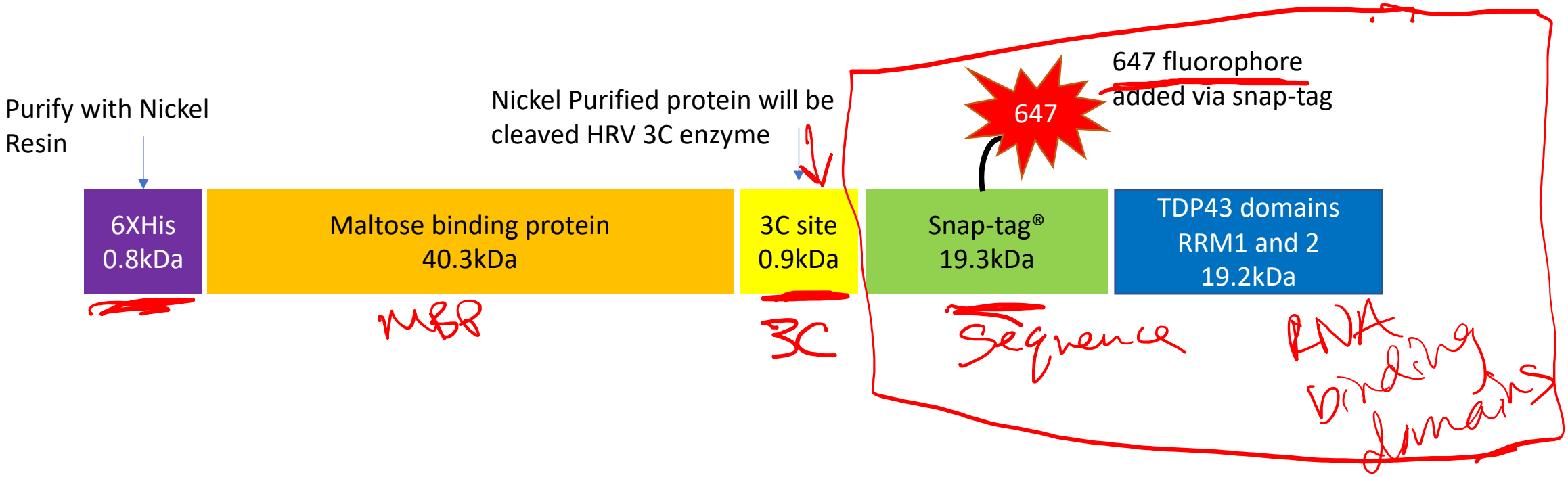
IPTG ‘induces’ protein expression by preventing LacI repression



pET_MBP_SNAP_TDP43-RRM12

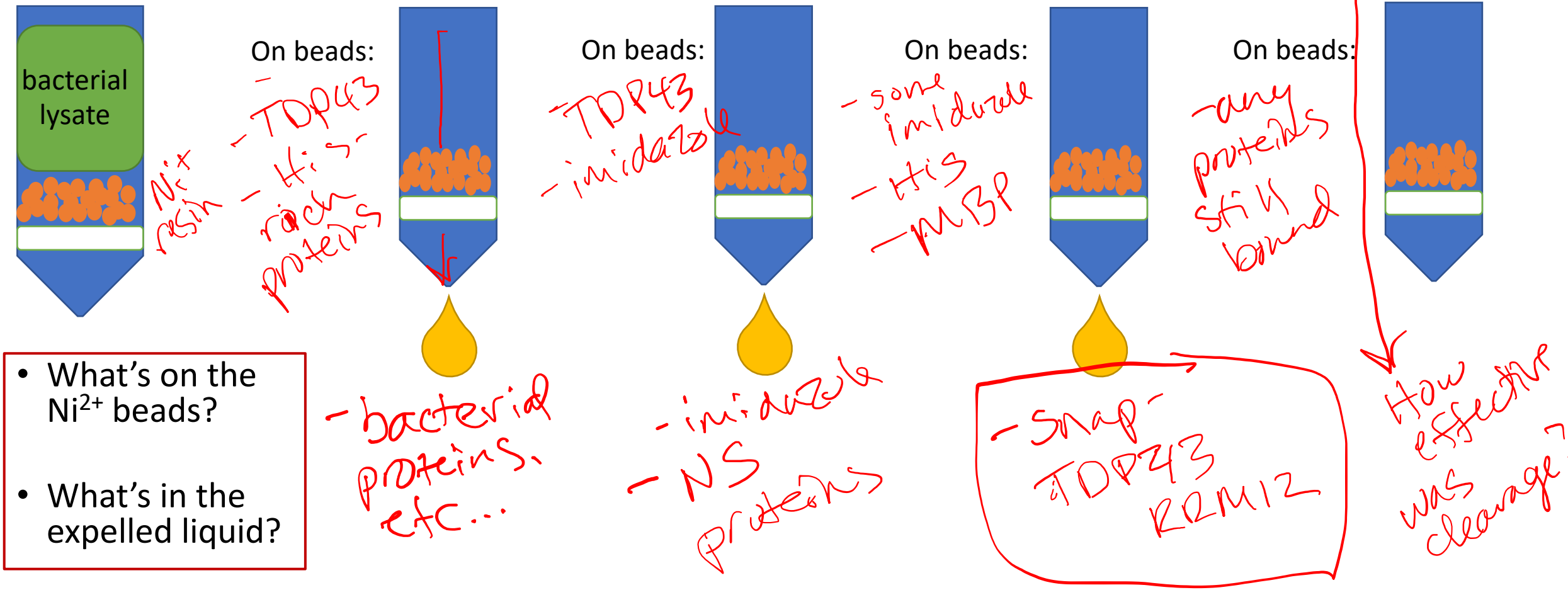
What is protein expressed in our system

Our protein for this module:



Protein purification review

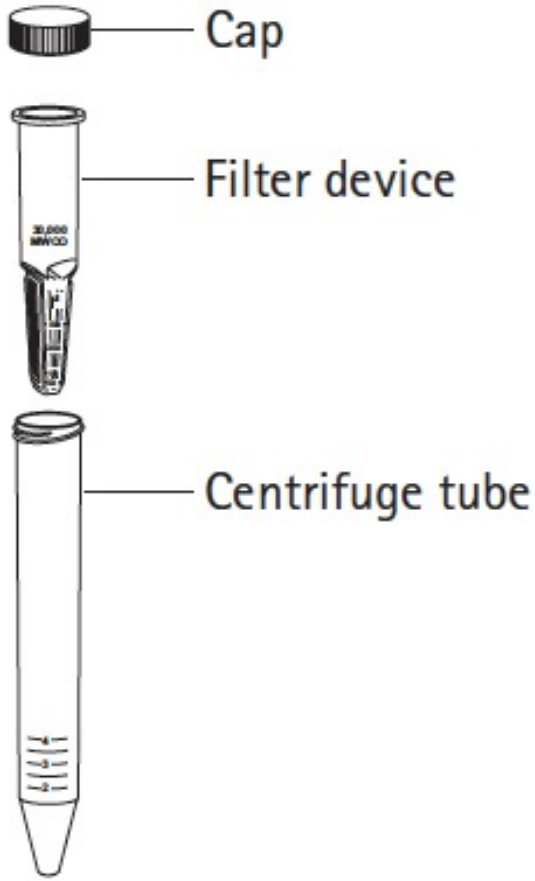
- Why this step?



- What's on the Ni^{2+} beads?
- What's in the expelled liquid?

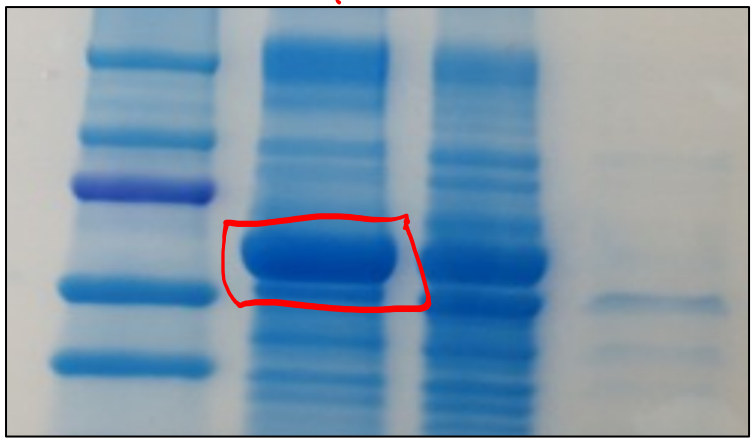
Protein is concentrated after purification

- Filter device sits within centrifuge tube
  - Protein added to filter device before centrifugation
- Filter has MW cutoff of 3 kDa
  - protein retained in the filter device during centrifugation
  - TDP43-RRM12 + Snap-tag = ?
  - 6x His tag = 2.5 kDa
- How does this concentrate the protein?

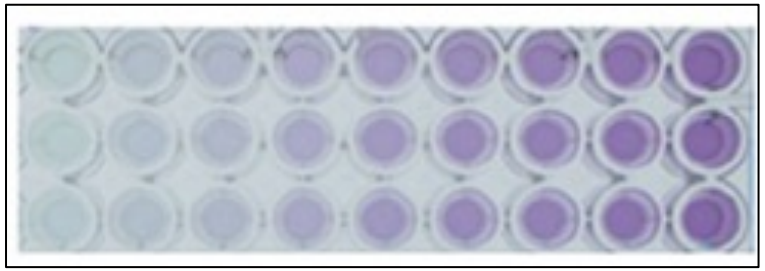


How will you assess purity and concentration?

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



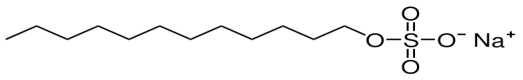
- Measure concentration using BCA assay
  - Colorimetric assay
  - Calculate concentration from standard curve



Bradford

Lowry

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

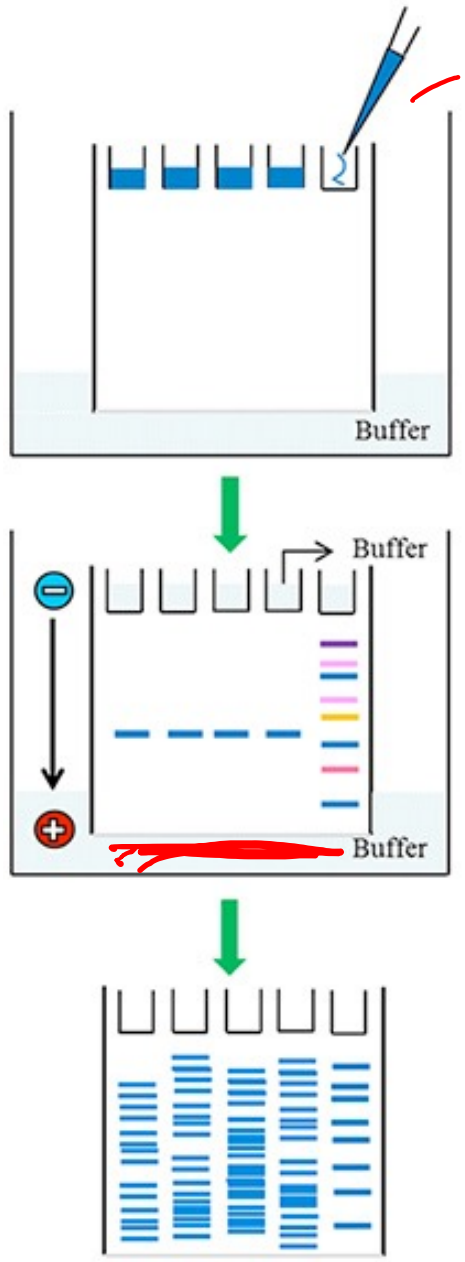


How are proteins separated?

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

- Running buffer

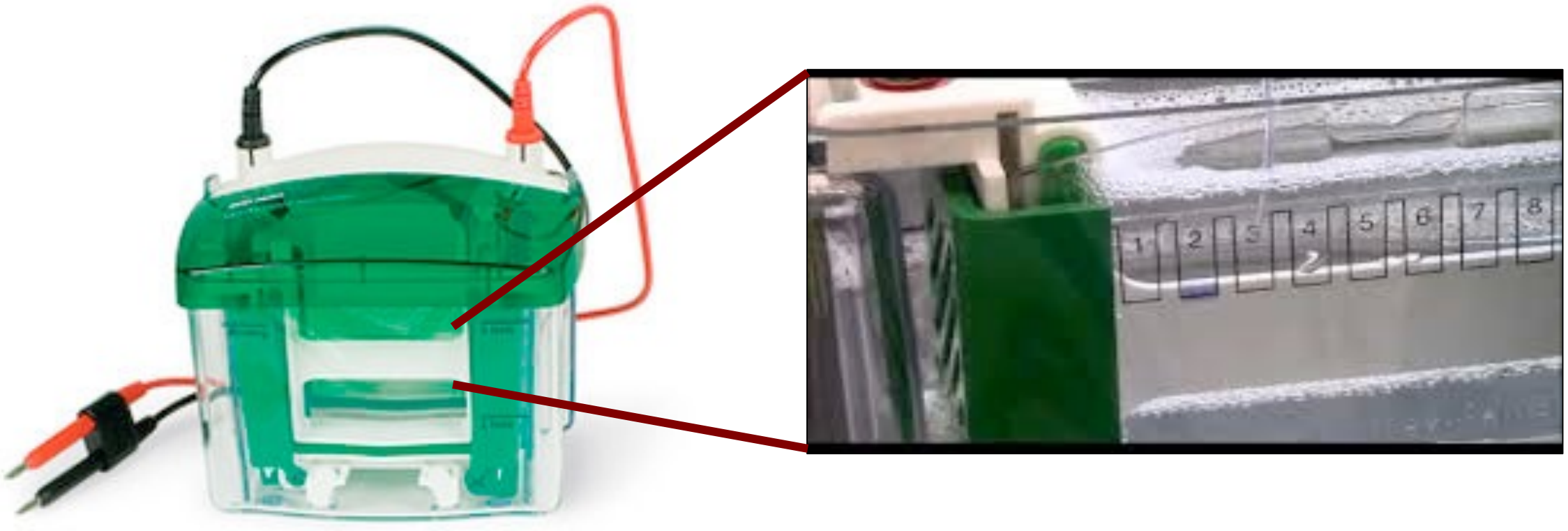
7 - regulate salts
- regulate pH



- sample buffer

Running to red

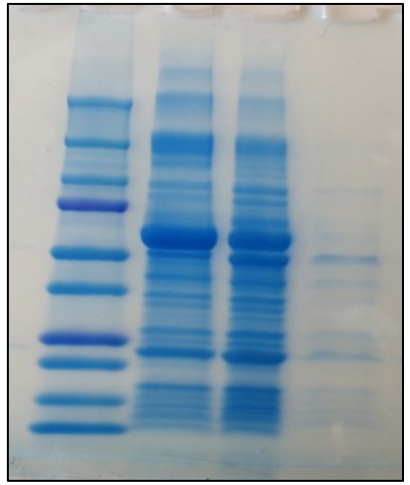
Demonstration of SDS-PAGE



How are proteins visualized?

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

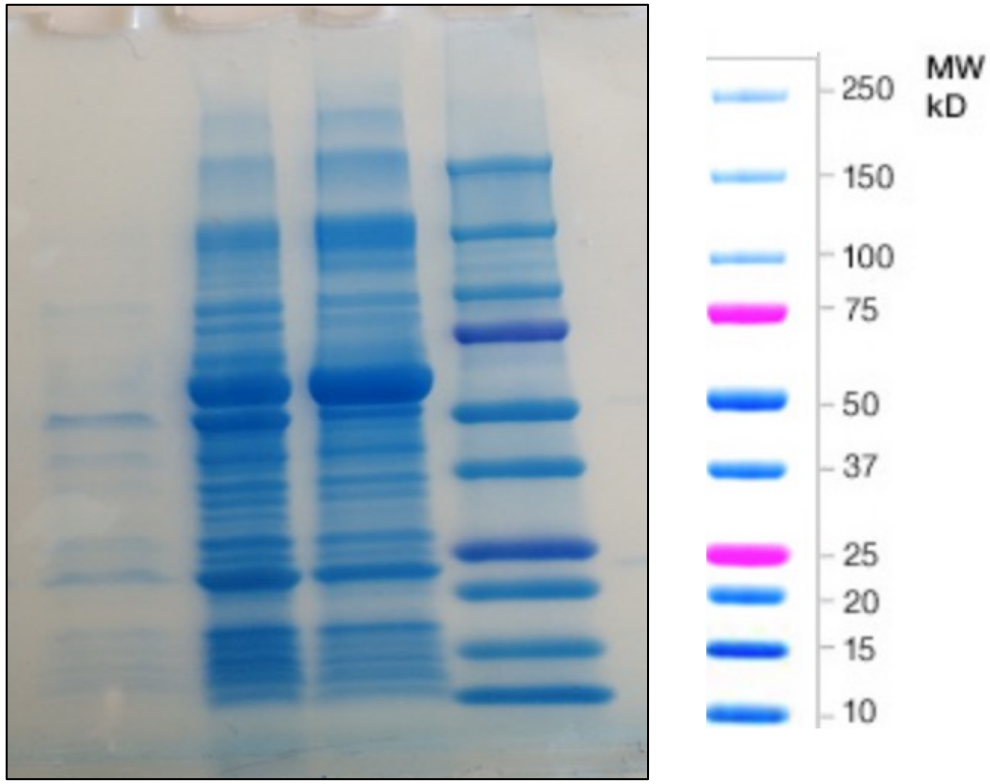
- Red if unbound (cationic form)
- Blue if bound to protein (anionic form)
- Hydrophobic and electrostatic interactions with basic residues
- Arg (also His, Lys, Phe, Trp)



What are the expected results of SDS-PAGE?

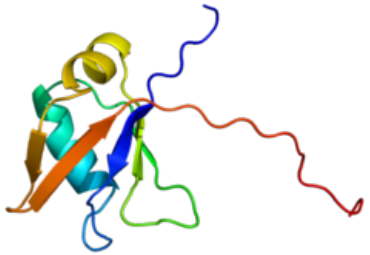
Each lane of the gel should be explained in the results

- 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+} → (OH^-, Temperature-dependent) → Cu^{1+}

- peptide bonds reduce copper ions
- proportional to [protein]

Step 2: BCA complexes with cuprous cation

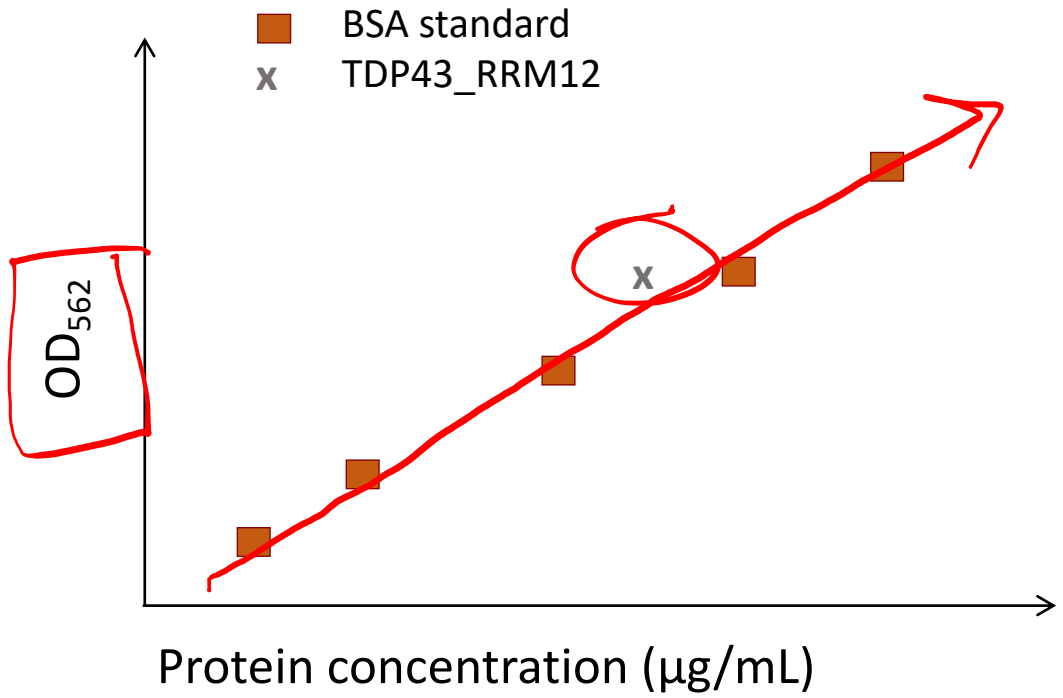
Cu^{1+} + 2 BCA → BCA-Cu^{1+} complex (A = 562 nm, violet)

- BCA chelate Cu^+ to complex

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

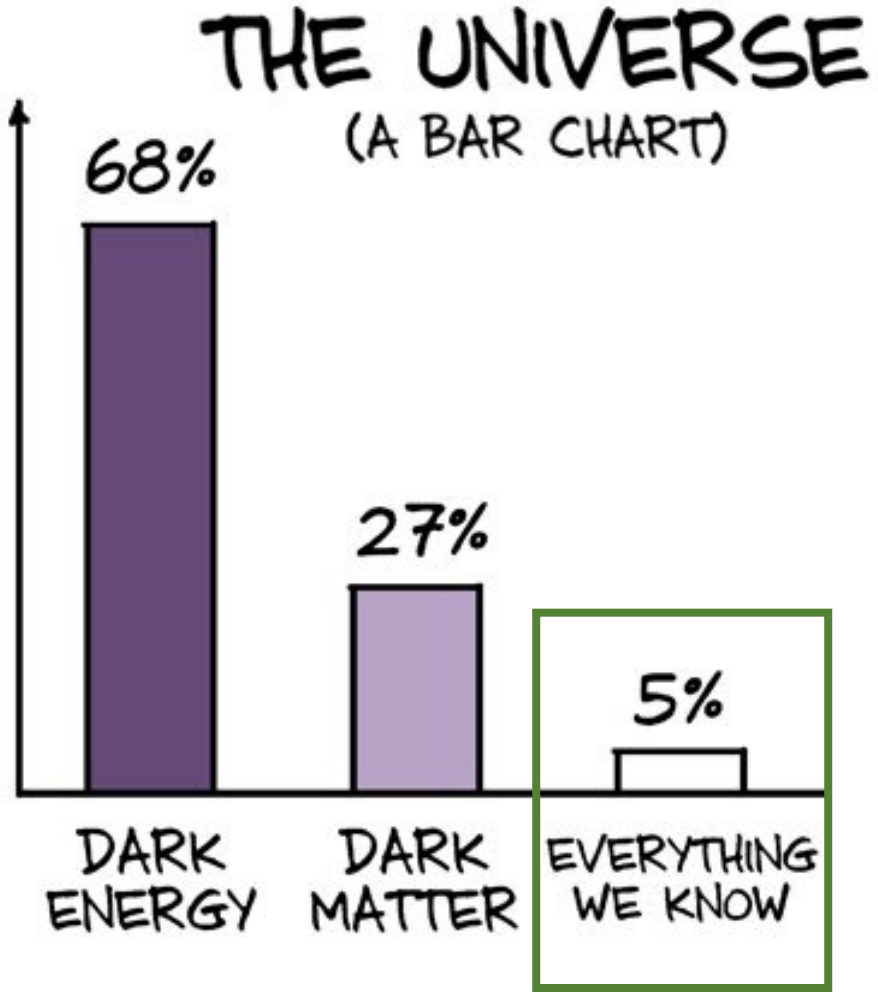
Standard curve generated using serial dilutions of bovine serum albumin (BSA)

- 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?



We know a small fraction of what the universe has to offer (a take-home message)

- Minimal text included to understand the figure
- Oooh, 5%. That's better than expected.
- But, what does that even mean?



phd comics

For today…

- Work through M2D2 laboratory exercises with partner

- Work on Mini-presentation!

For M2D3…

- Outline the Introduction section for Research article
- Review paper for in-class discussion with partner,
  - Draft slide, script for presenting Figure 1 from that paper