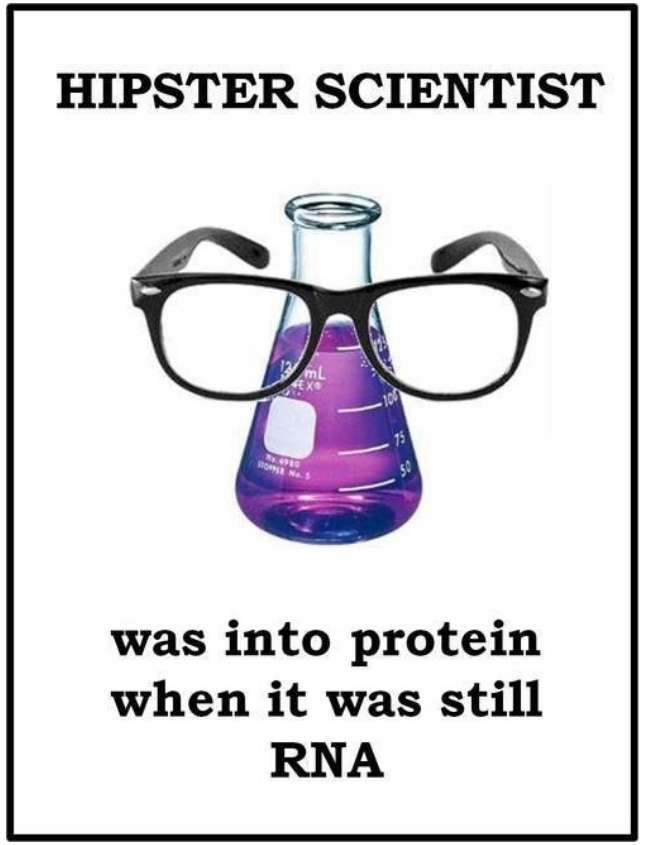
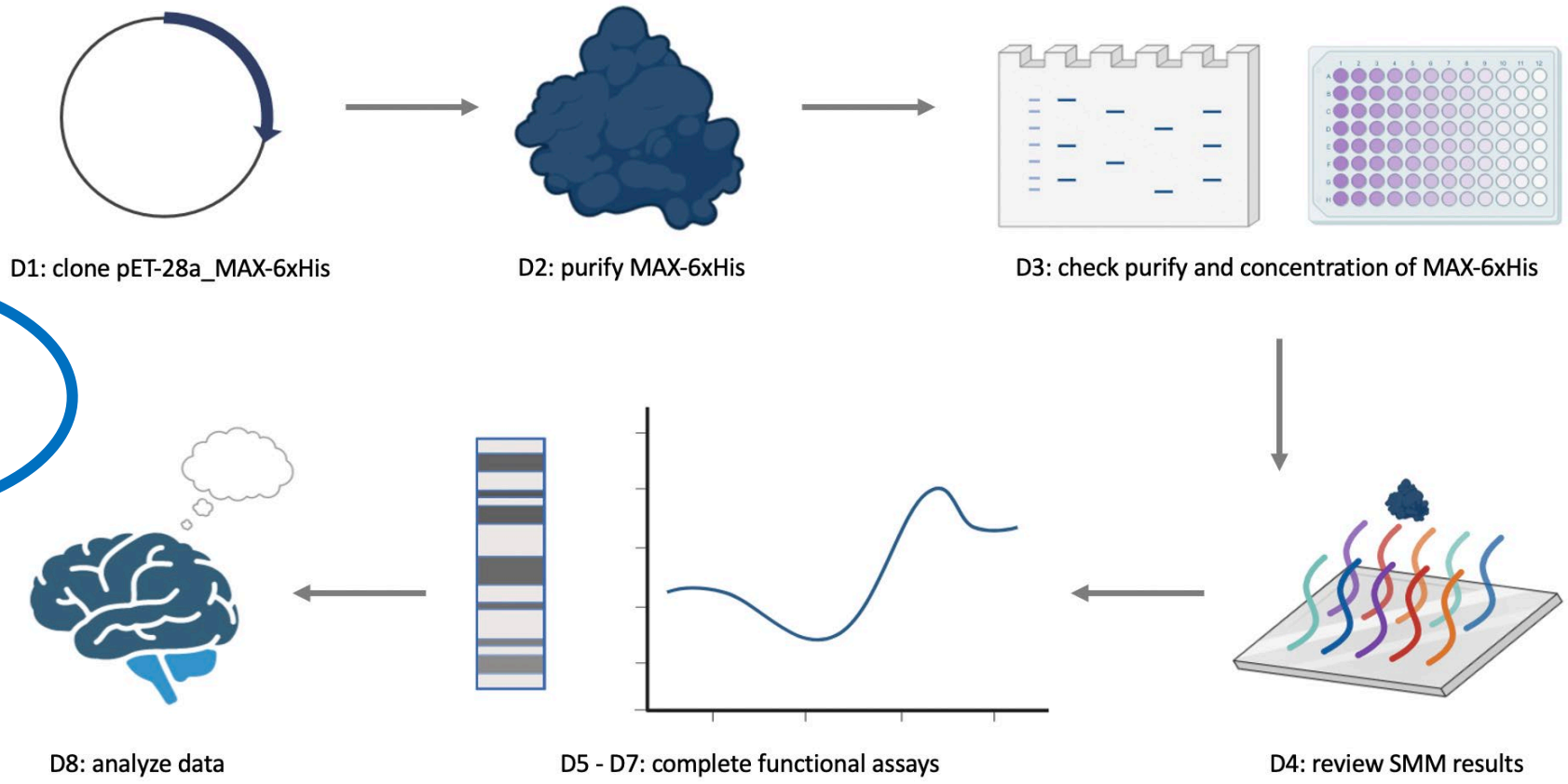
M1D3:
Assess purity and concentration of purified protein

1. Prelab discussion
2. Concentrate purified protein sample
3. Visualize MAX-6xHis purity
4. Measure MAX-6xHis concentration



Overview of Mod 1 experiments:



Let's review the protein purification steps…

- Added lysonase – to what? why? Added DNaseI – to what? why?

  Lysonase contains enzymes that break down bacterial cell walls – DNAse prevents clumping

- Centrifuged – what? why?

  Centrifuging the lysed cells separates cell debris from lysate

- Incubated with nickel resin – why?

  Lysate contains our POI, which has a his tag that likes to bind nickel

- Washed with low concentration imidazole – why?

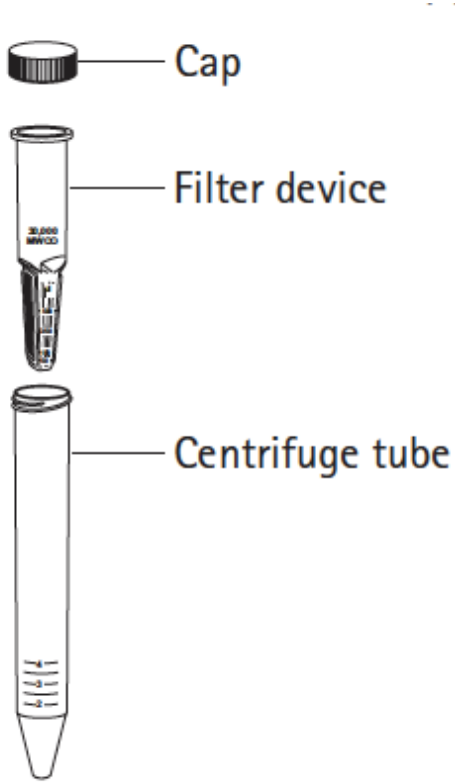
  Low imidazole competes with weak binders for binding to the nickel column

- Eluted with high concentration imidazole – why?

  High imidazole competes with our POI for binding to the nickel column

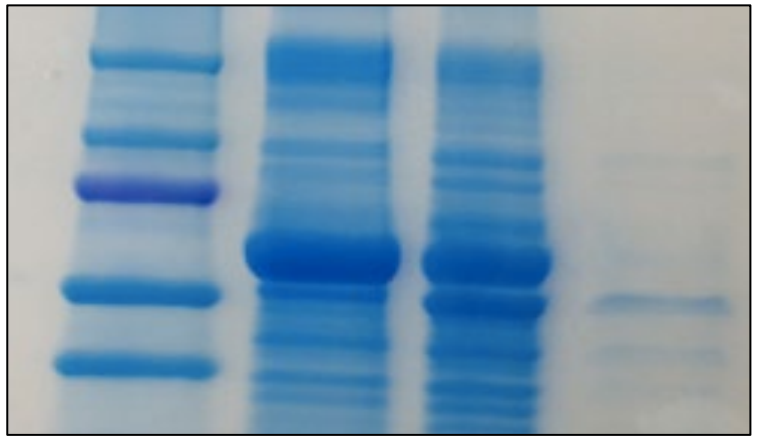
First, you will concentrate your purified protein

- 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
- How does this concentrate the protein?

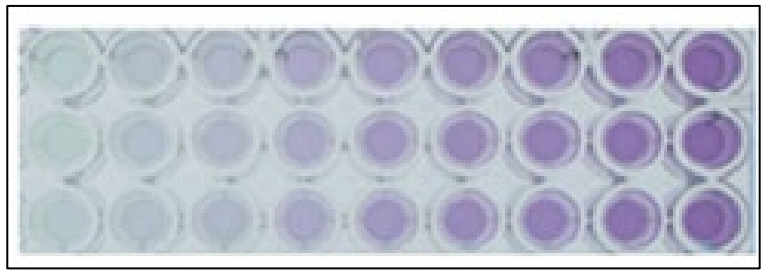


How will you assess the quality of your protein?

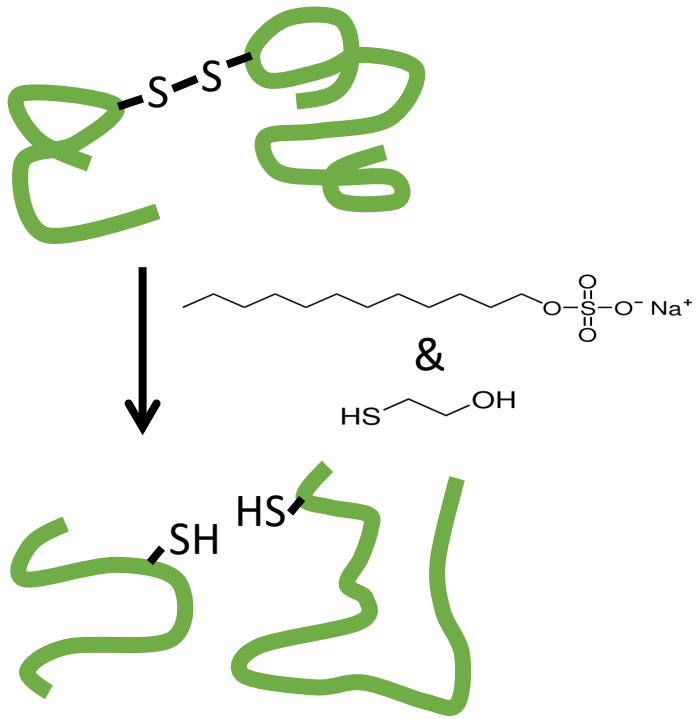
- Check purity using SDS-PAGE
  - Visual detection of all proteins in sample
  - Used to assess purity / quality



- Measure concentration using BCA assay
  - Quantitative measure of all proteins in the sample
  - Used to calculate concentration



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

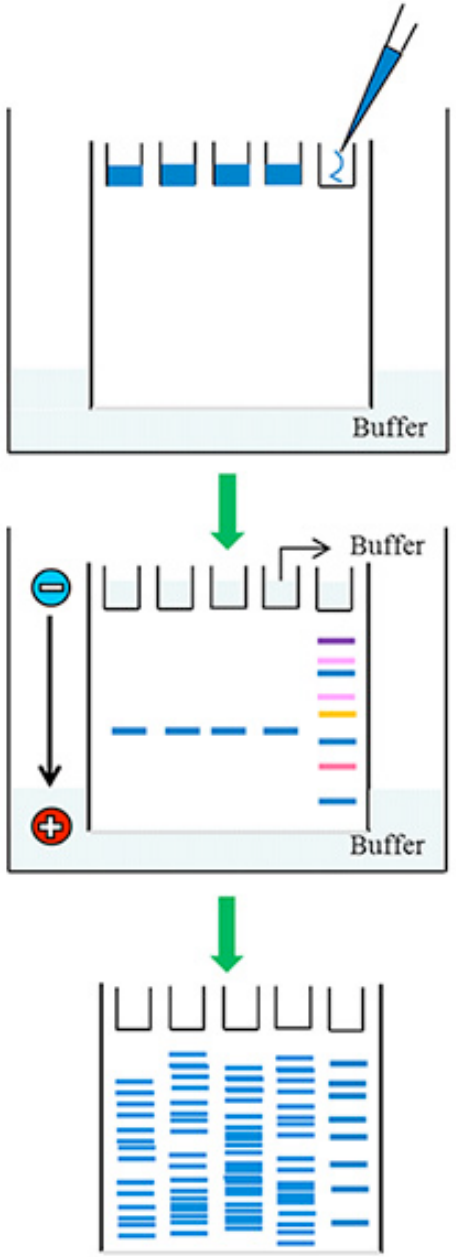


- Laemmli sample buffer (loading dye):
  - sodium dodecyl sulfate (SDS)
  - β-mercaptoethanol (BME)
  - bromophenol blue
  - glycerol
- Boiling:

How are proteins separated?

- Electrophoresis completed in TGS buffer
  - Tris-HCl
  - SDS
  - Glycine

- How does adding Laemmli buffer and boiling change protein structure?
- What determines how far a protein migrates in the gel?



Be mindful when loading protein samples!

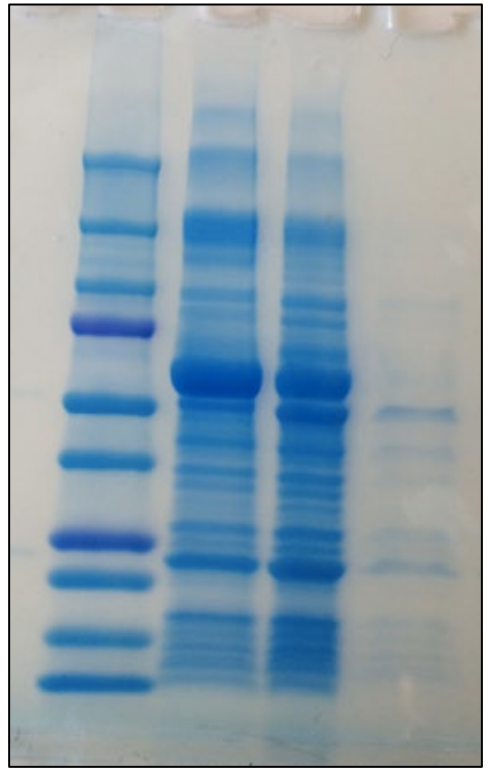
- Consider the order of your samples:
  - Pellet
  - Lysate
  - Flowthrough
  - Wash
  - Concentrated MAX-6xHis
  - Slurry
  - Ladder

- Figure for these results will be used for your due M1D4 homework and included in your Data summary!

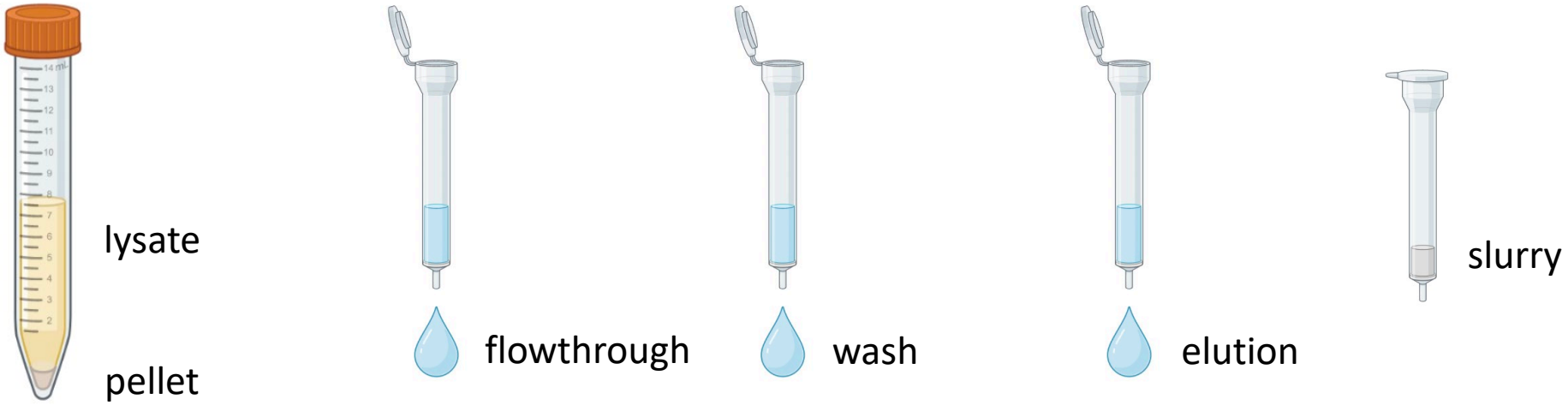
How are proteins visualized?

- Coomassie dye used to stain protein bands
  - Hydrophobic and electrostatic interactions with basic residues
  - Arg (also His, Lys, Phe, Trp)

- How will you know which band corresponds to MAX-6xHis?
- How does SDS-PAGE provide information regarding the purity of your protein sample?



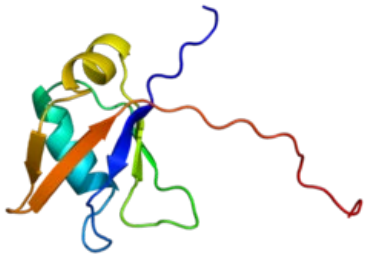
What are the expected results?



- Where do you / do you not expect to see MAX-6xHis? Why / why not?
- Where do you expect to see other cellular proteins?
- How will the elution sample differ from the concentrated protein sample?

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+}

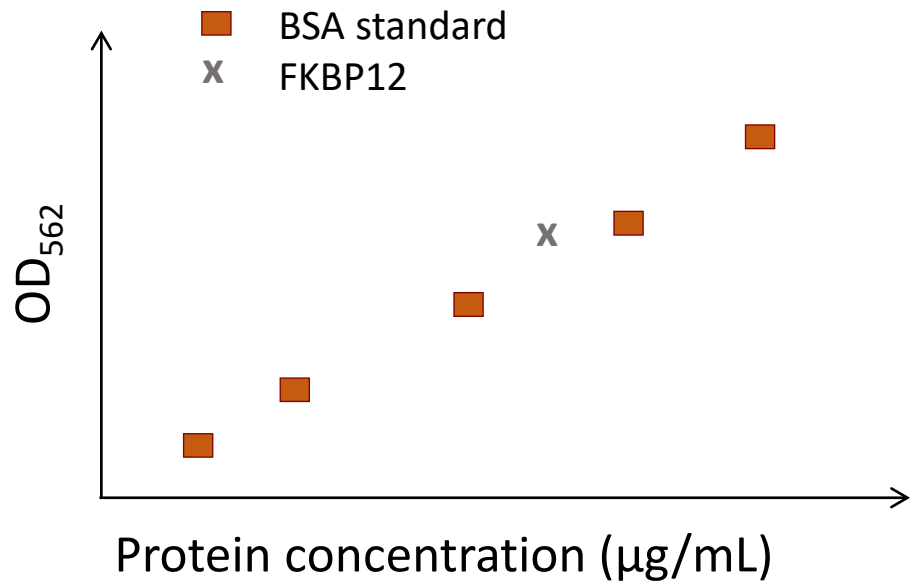
Step 2: BCA complexes with cuprous cation

Cu^{1+} + 2 BCA $\longrightarrow$ BCA-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 sure to add correct volumes

- Is the calculated concentration an accurate measure of the amount of MAX-6xHis in your sample?



For today…

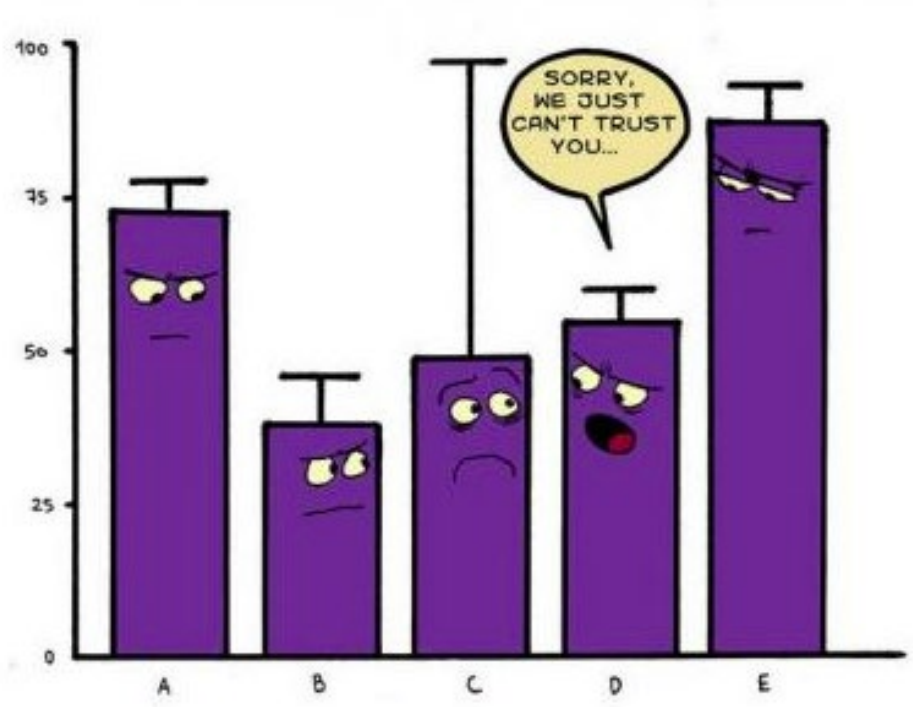
- Divide experiments between partners to ensure work is completed during class time
  - Be sure to share data / results!

For M1D4…

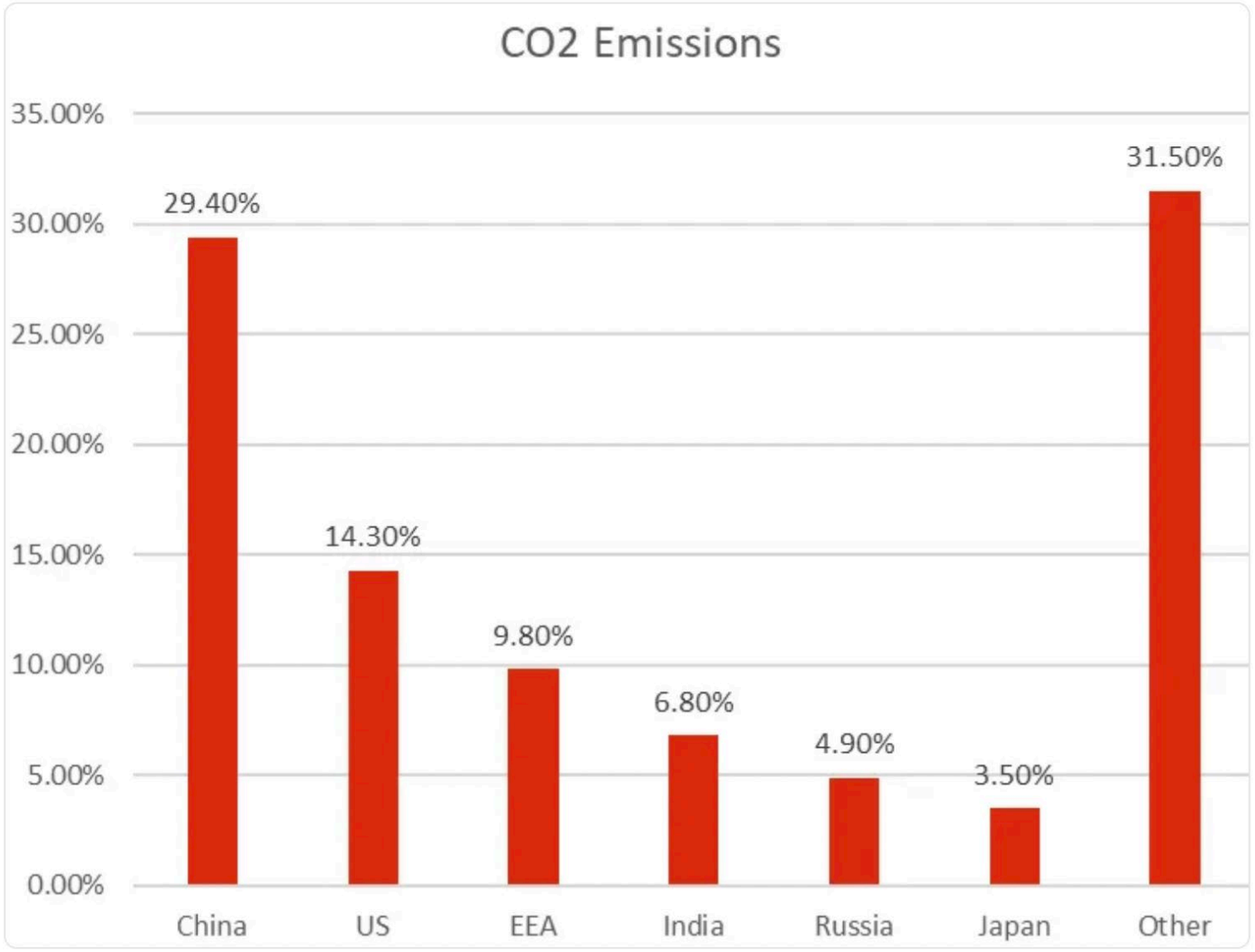
- Craft data figure for SDS-PAGE and BCA results
- *Draft outline of Background & Motivation for bonus feedback* ☺

Notes on figure making…

- Be sure image is appropriately sized
  - Only needs to be large enough to be legible
  - Should not be entire page
- Eliminate visual noise and clutter
  - Unnecessary labeling and graphics distract from the data
- Use clear labels / legends
  - Be sure labels / legends do not obstruct the data



How can you improve this example?



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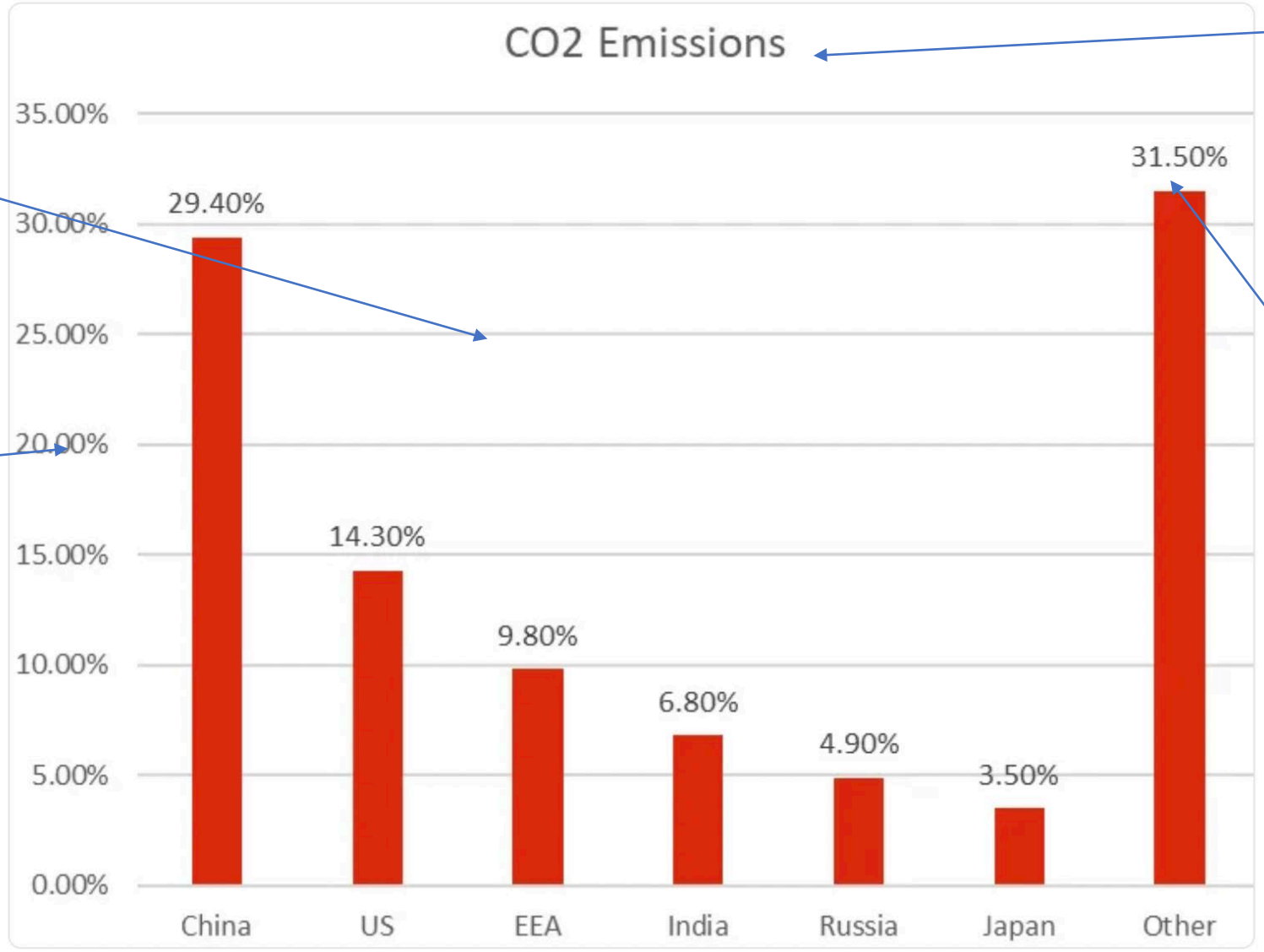

Title should be in the caption. This would be better as an axis label

Gridlines not necessary

No need for this level of precision in the axis

Explicit percentages can be referred to in the body of the text