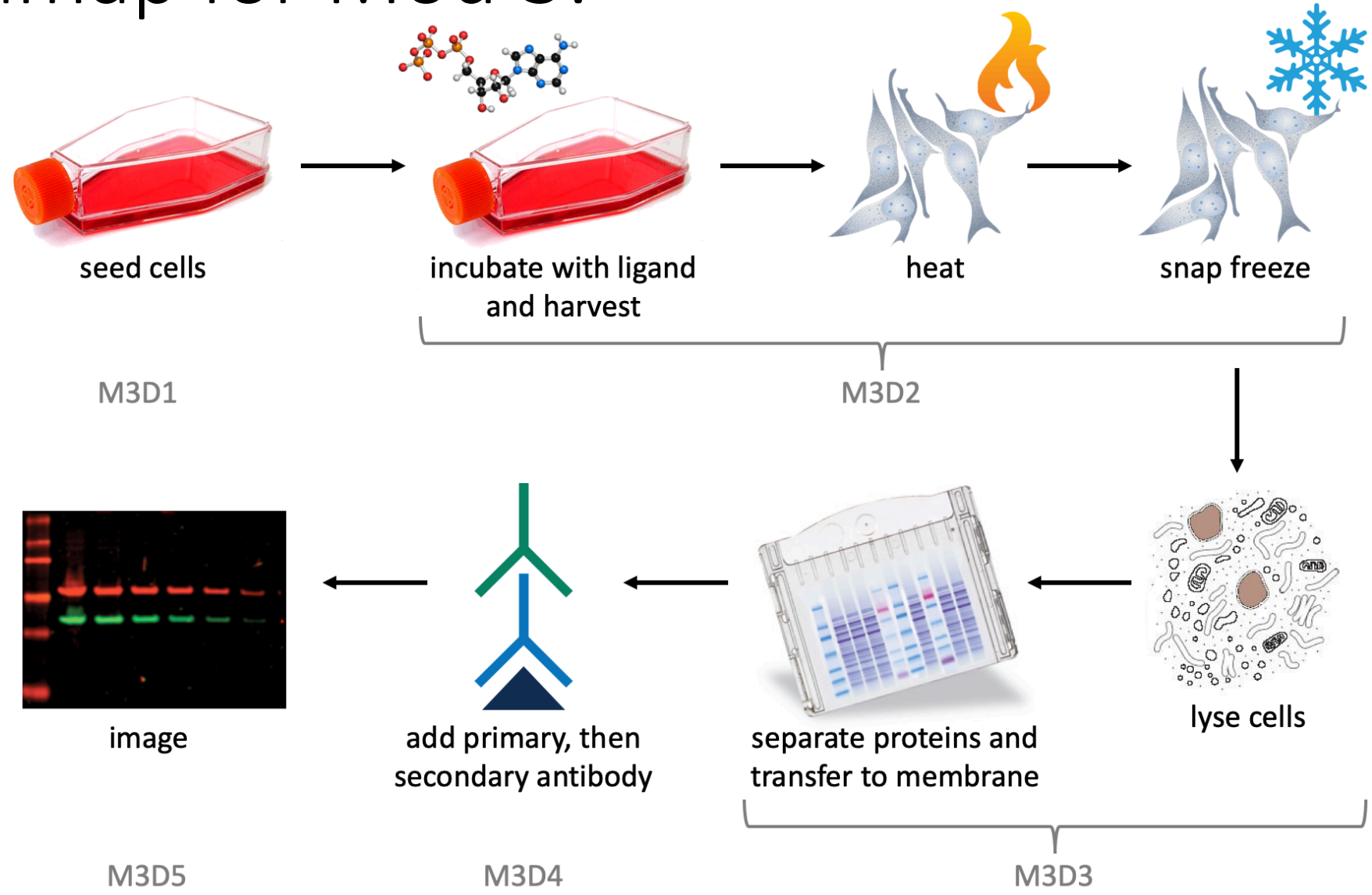
M3D3:
Begin Western blot analysis

QUIZ

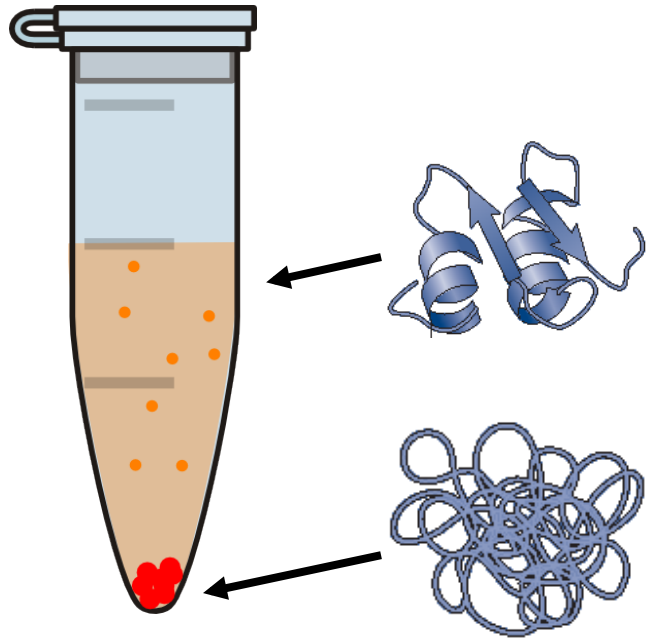
1. Prelab discussion
2. Prepare cell lysate
3. Separate cellular proteins
4. Transfer proteins to membrane

Roadmap for Mod 3:



Prepare cell lysate by removing protein aggregates

- In the previous class, cells were treated with ligand and heated to denature protein
- During snap-freeze / thaw cycles, cell membranes fractured and weakened
- Today, lysate will be centrifuged to pellet protein aggregates and cell debris
  - Soluble protein will be used for CETSA

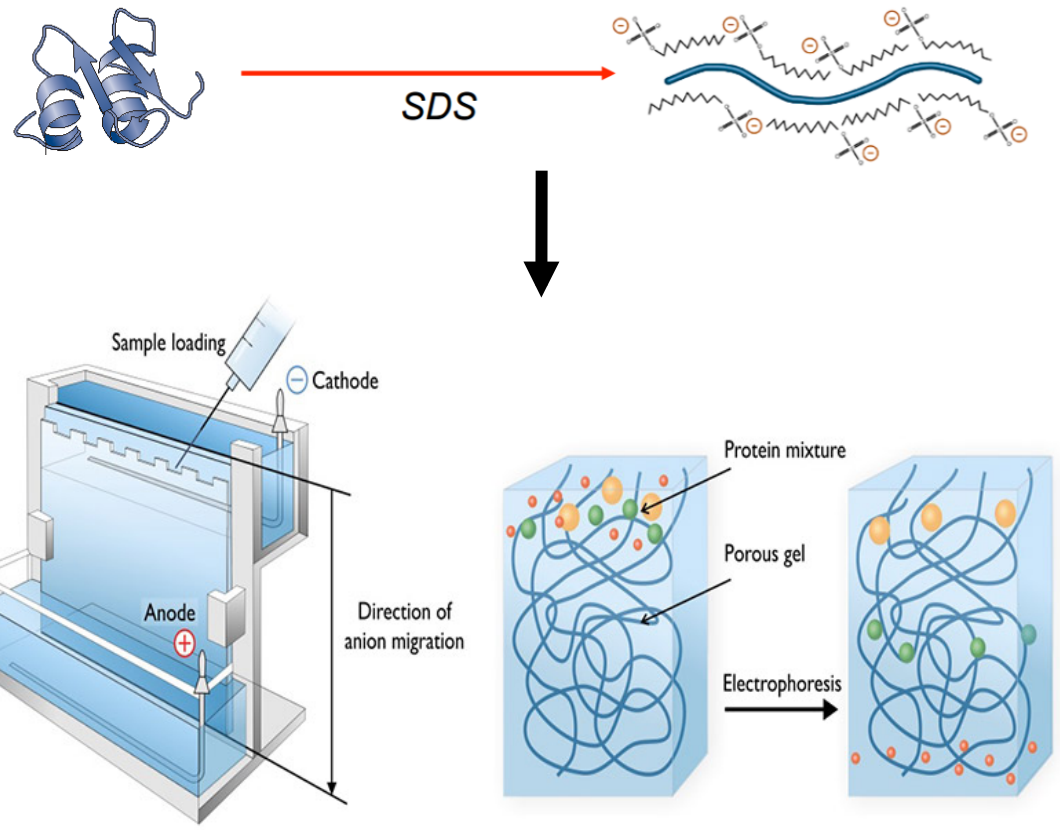


Denature soluble proteins for CETSA analysis

- Laemmli buffer added to prepare proteins for electrophoresis
  - Tris
    Salts, pH
  - SDS
    coats protein → net negative charge
  - glycerol
    Weights Sample
  - BME
    breaks disulfide bonds
  - bromophenol blue
    dye front

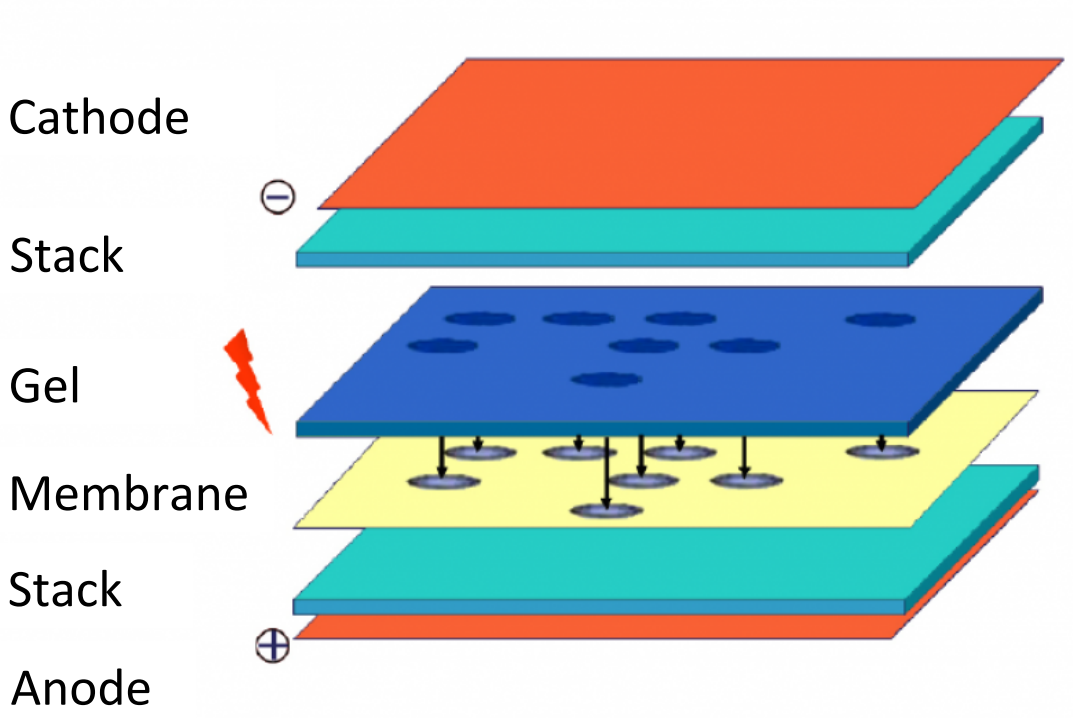
Separate cellular proteins using polyacrylamide gel electrophoresis (PAGE)

- Because charge and secondary structure are alleviated, SDS-PAGE separates proteins according to size
- As with agarose electrophoresis, molecules migrate toward positive electrode (anode)



Transfer proteins to membrane for Western blot analysis

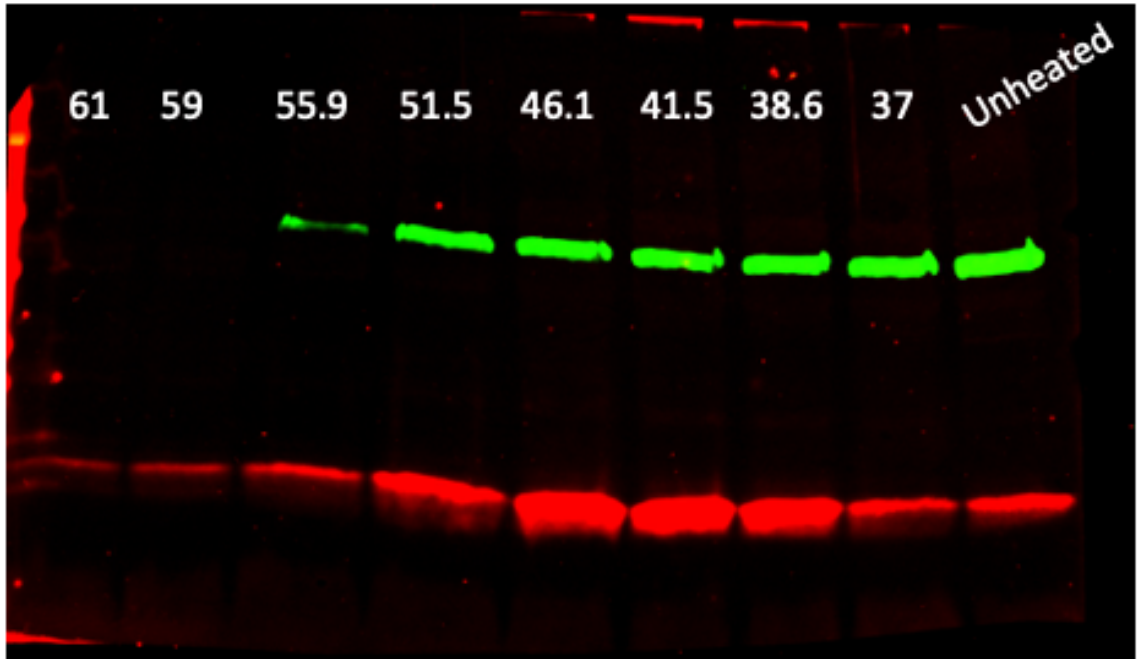
- Polyacrylamide gels are fragile and prevent further analysis of protein composition
- Again, net negatively charged proteins are migrated using a current from the gel to a membrane
- Membrane can be blotted to determine presence of protein of interest



CETSA visualized via Western blot

- Membrane with proteins is ‘blotted’ using antibodies to probe for specific for protein of interest

- Why do we need to probe for our protein of interest?
- Why do we probe for tubulin?



For today…

- Use downtime to work on your Research proposal idea!

For M3D4…

- With your partner, create a Research page to organize your ideas / thoughts
  - See prompts on wiki!
- With your partner, outline Background / Approach section of Mini-report
  - Review Mini-presentation assignment page on wiki