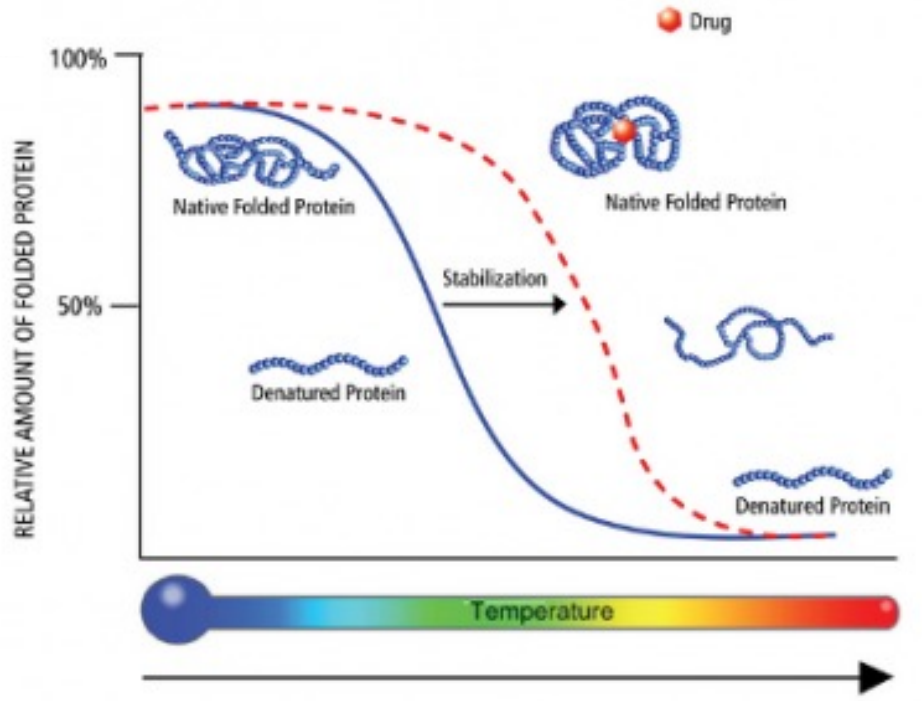
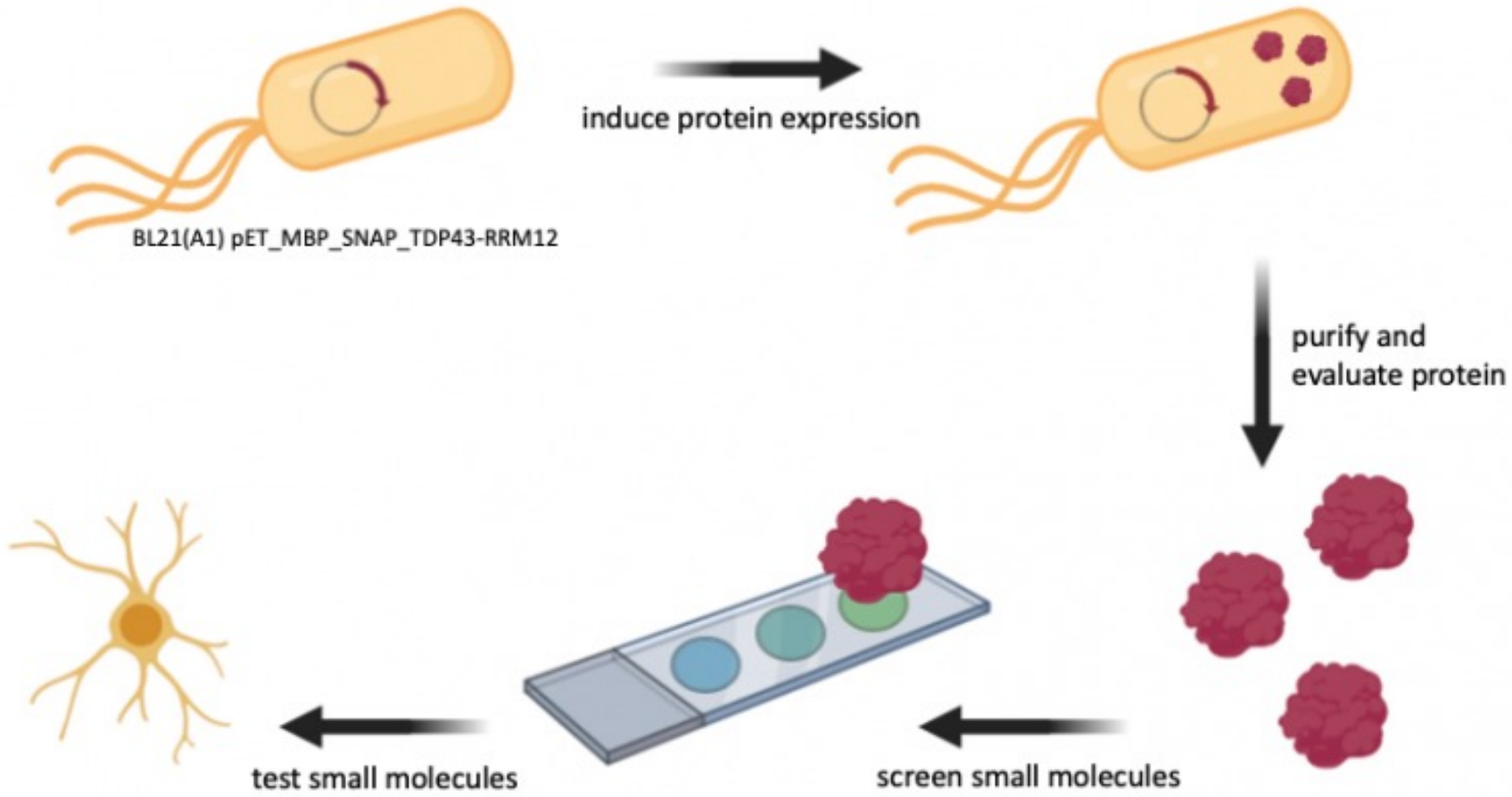
M2D6: Utilize cellular thermal shift assay (CETSA) to test putative small molecule binders

1. Prelab Discussion
2. Treat cells with small molecule ligands
3. Run SDS-PAGE
4. Protein gel transfer for Western Blot



Mod2 Overview



Cellular thermal shift assay (CETSA)

PROTOCOL

The cellular thermal shift assay for evaluating drug target interactions in cells

Rozbeh Jafari[1], Helena Almqvist[2], Hanna Axelsson[2], Marina Ignatushchenko[1], Thomas Lundbäck[2], Pär Nordlund[1] & Daniel Martinez Molina[1]

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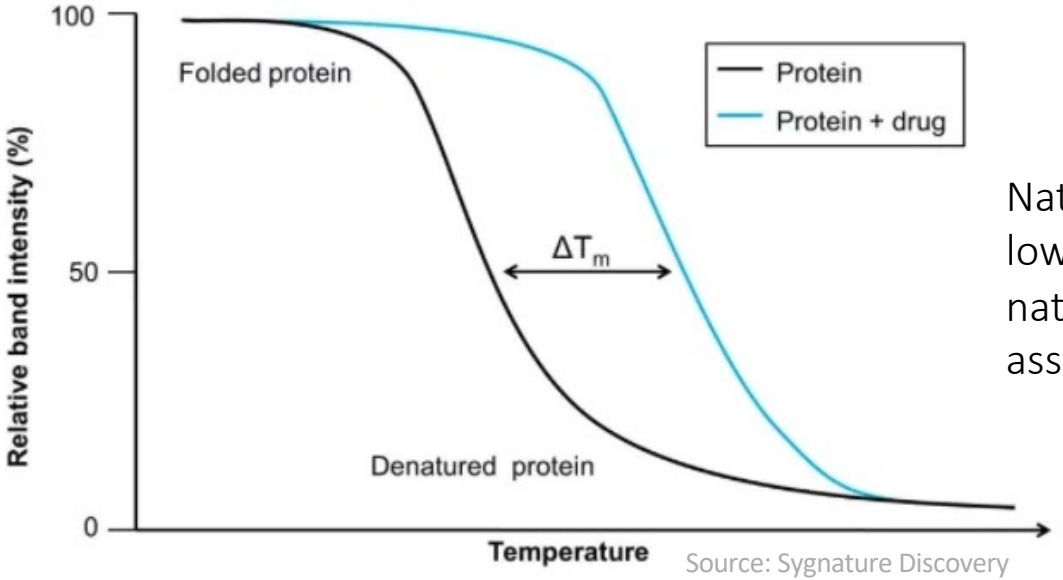

Native proteins denature at lower temperature than native folded proteins associated with drug

Source: Sygnature Discovery

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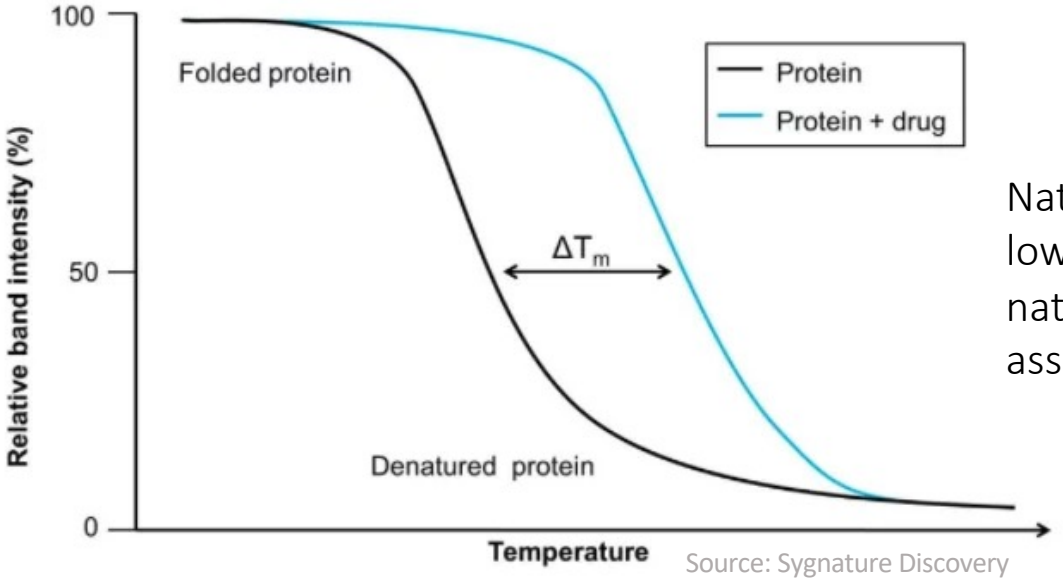

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- The ΔT_m indicates protein stabilization / destabilization compared to control

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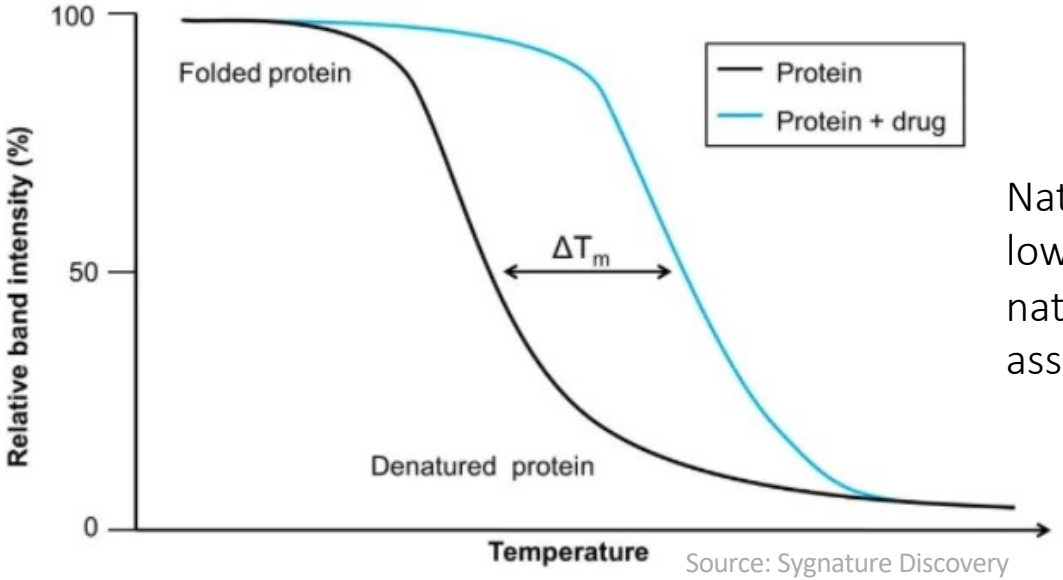

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- Assesses thermal stabilization of protein in presence / absence of ligand **in the cell**

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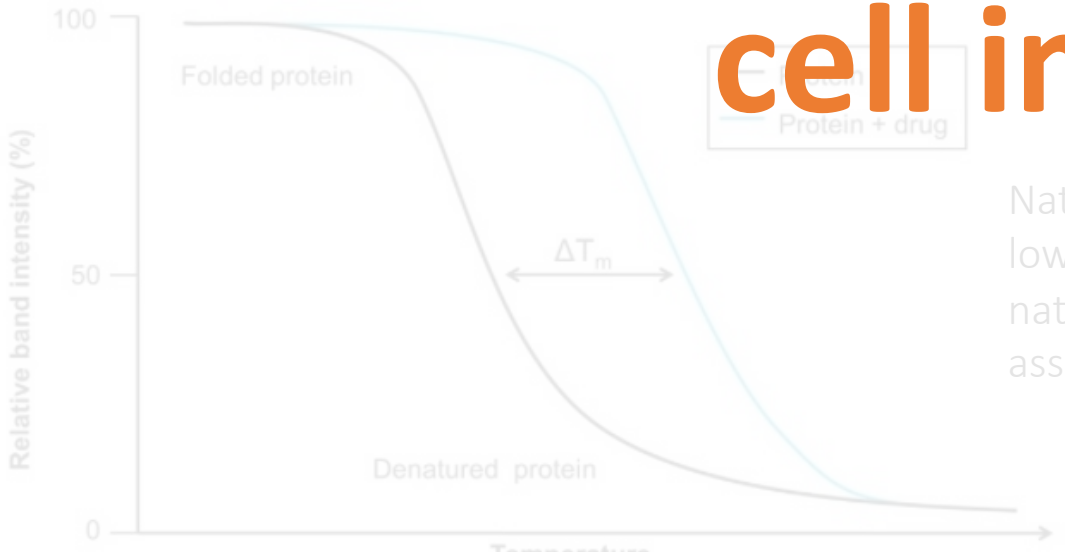
Why is testing for ligand binding in a cell important?



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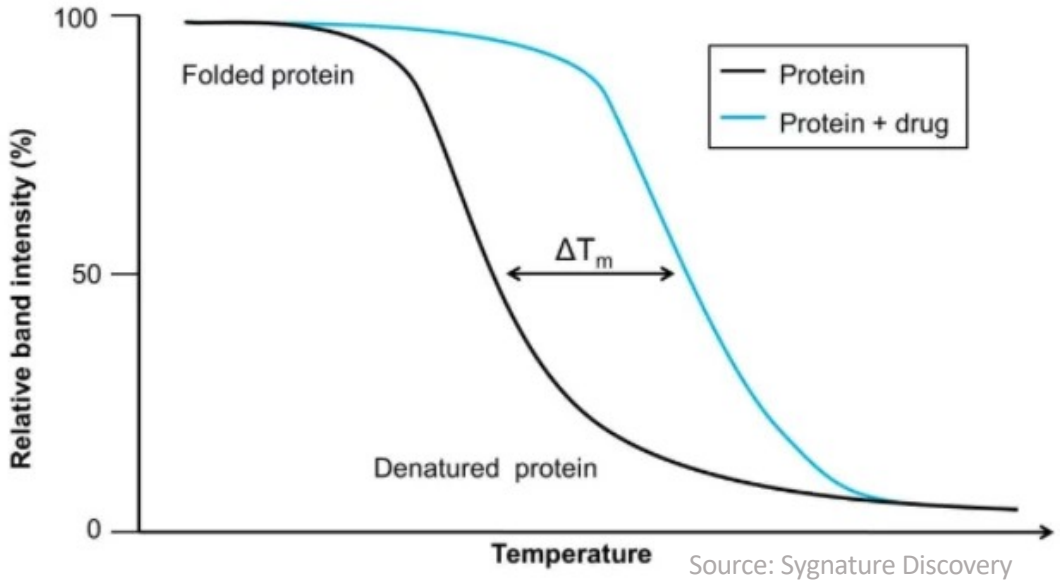
CETSA Overview



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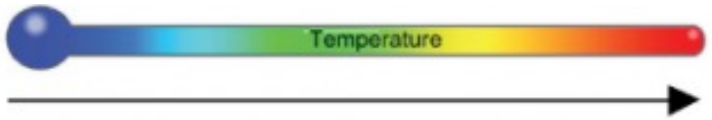
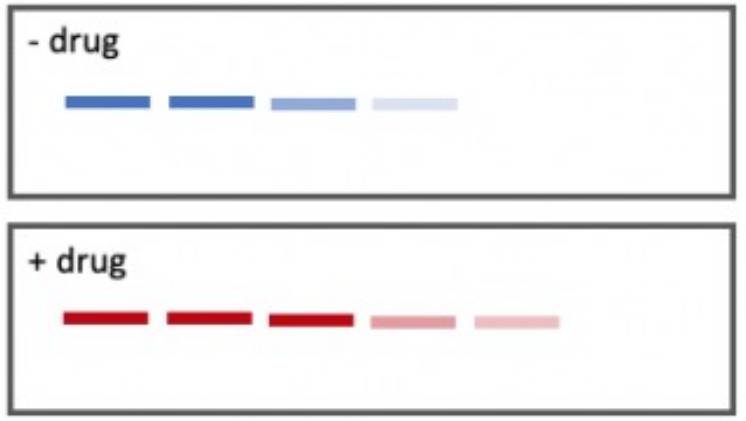
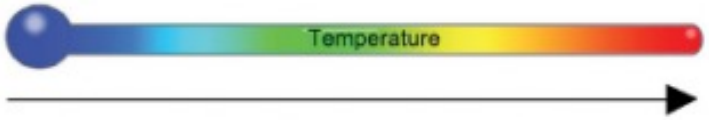


Identify presence of native folded proteins





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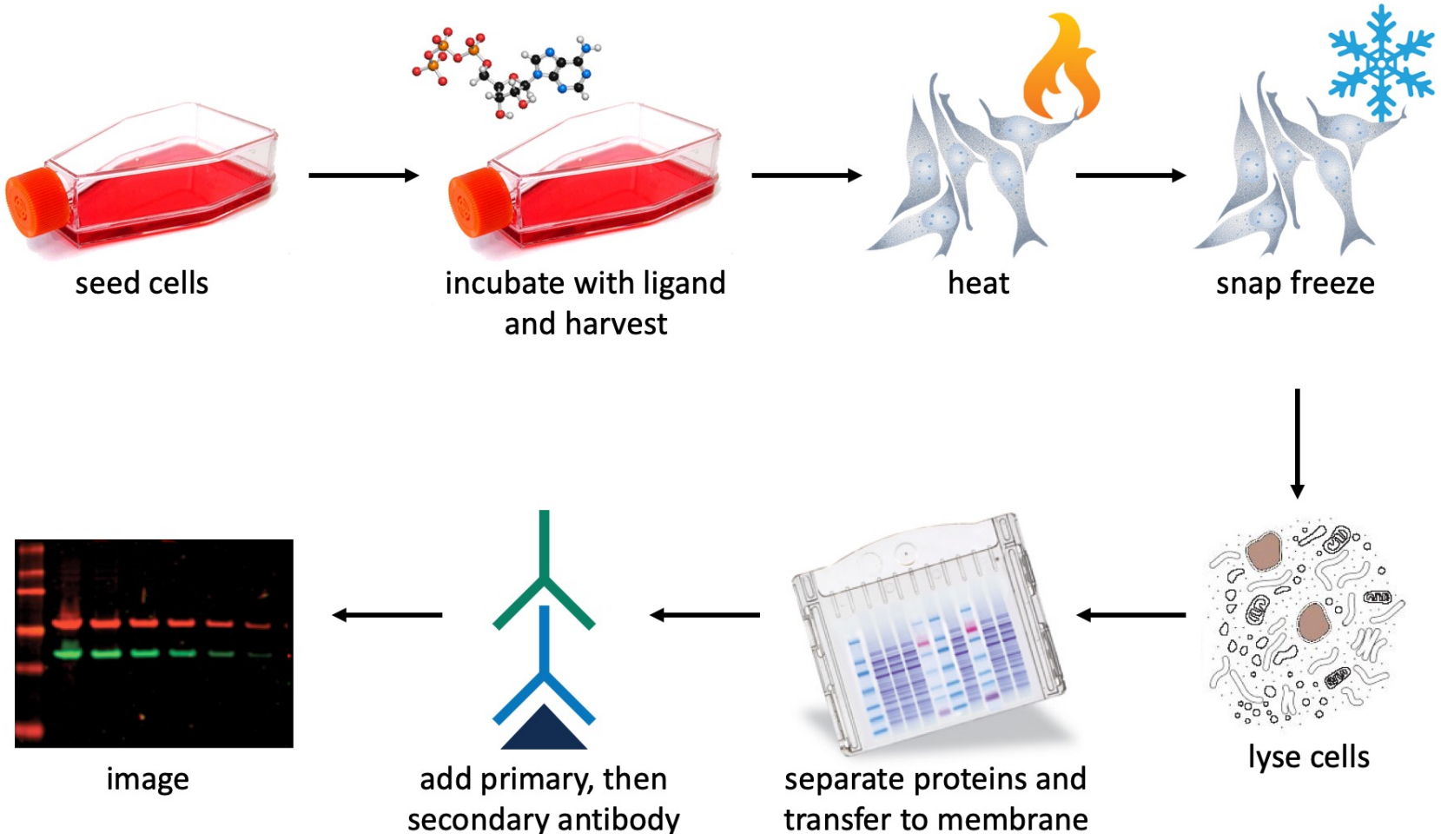
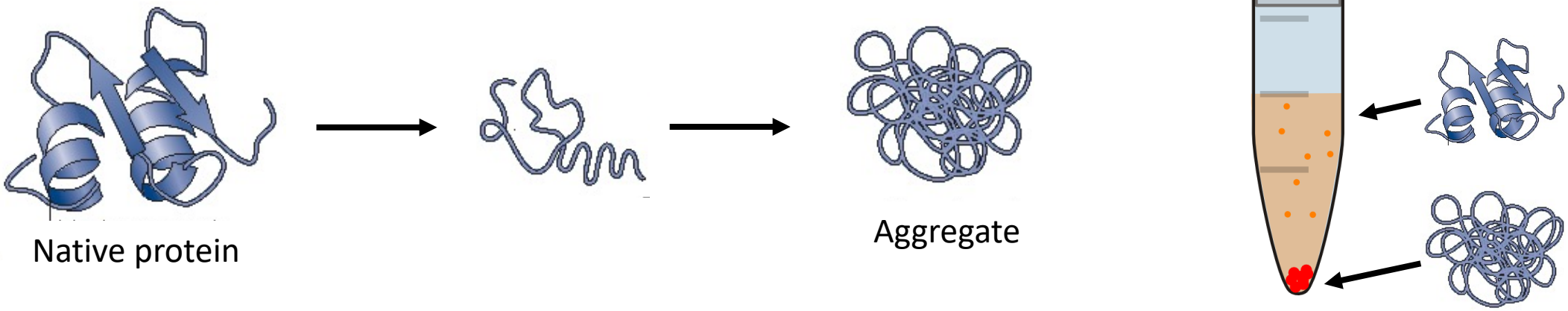


Heat causes protein denaturation

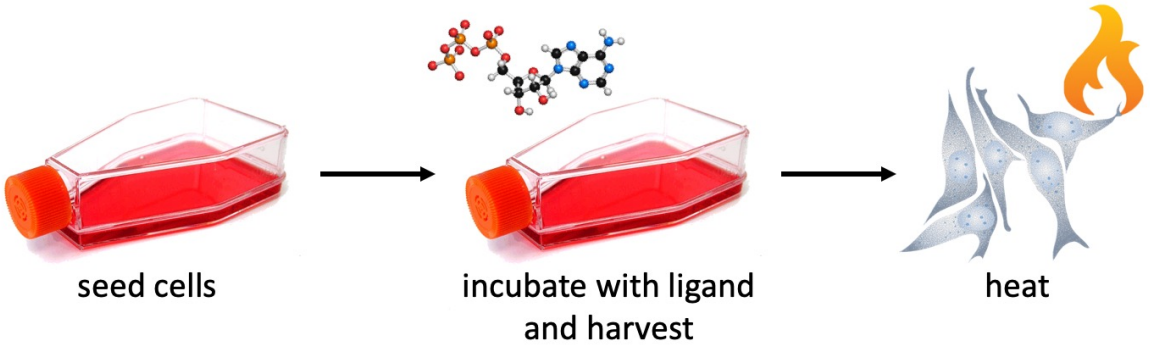
- As proteins denature, ‘melted’ primary structures aggregate
- Aggregates precipitate out of solution and can be removed via centrifugation



Treat Cells for CETSA

- **Heat:**

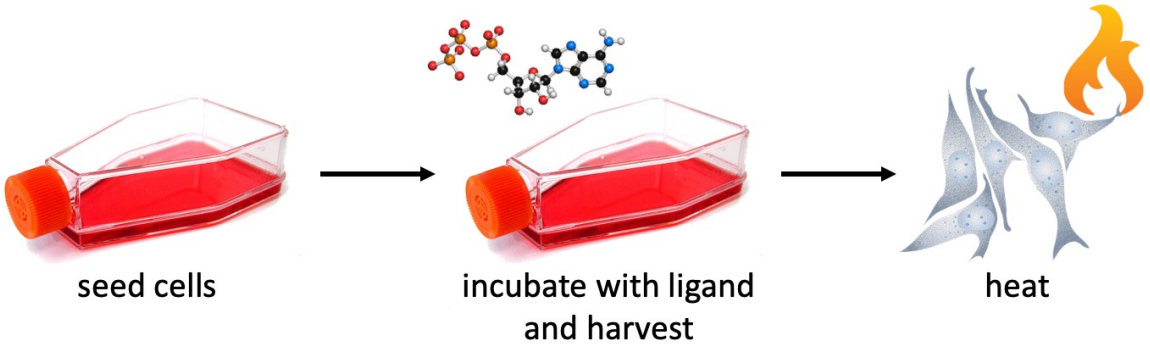
- **Snap freeze:**



Treat Cells for CETSA

- **Heat:** Test protein stability and remove denatured proteins later

- **Snap freeze:**



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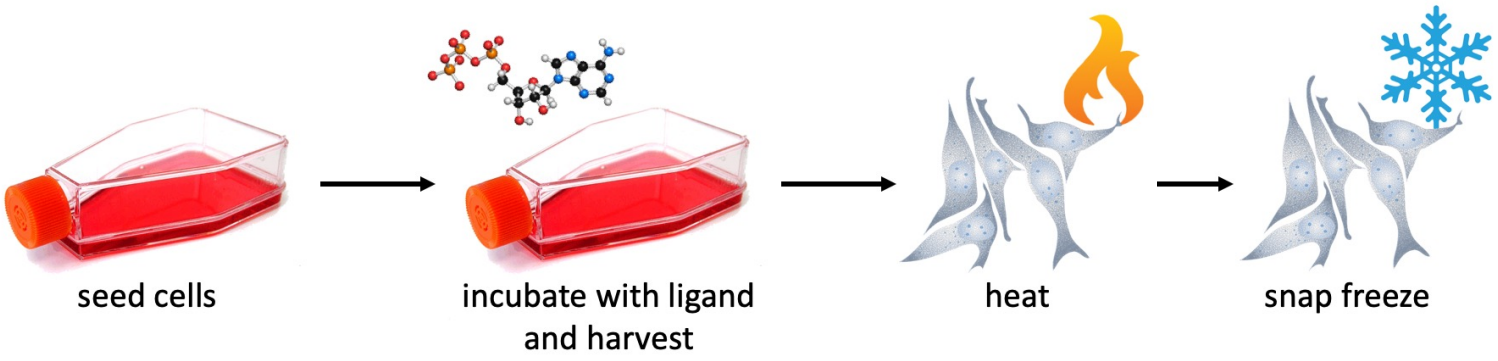
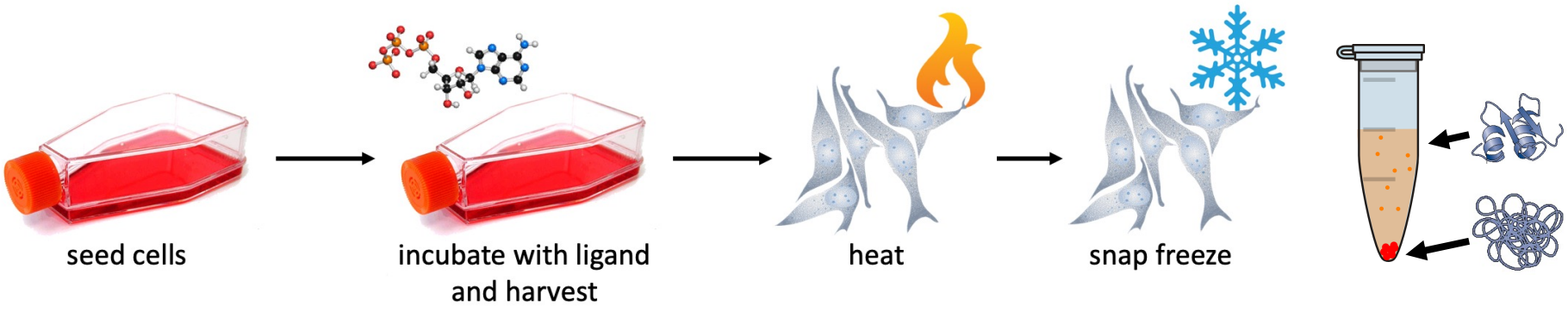
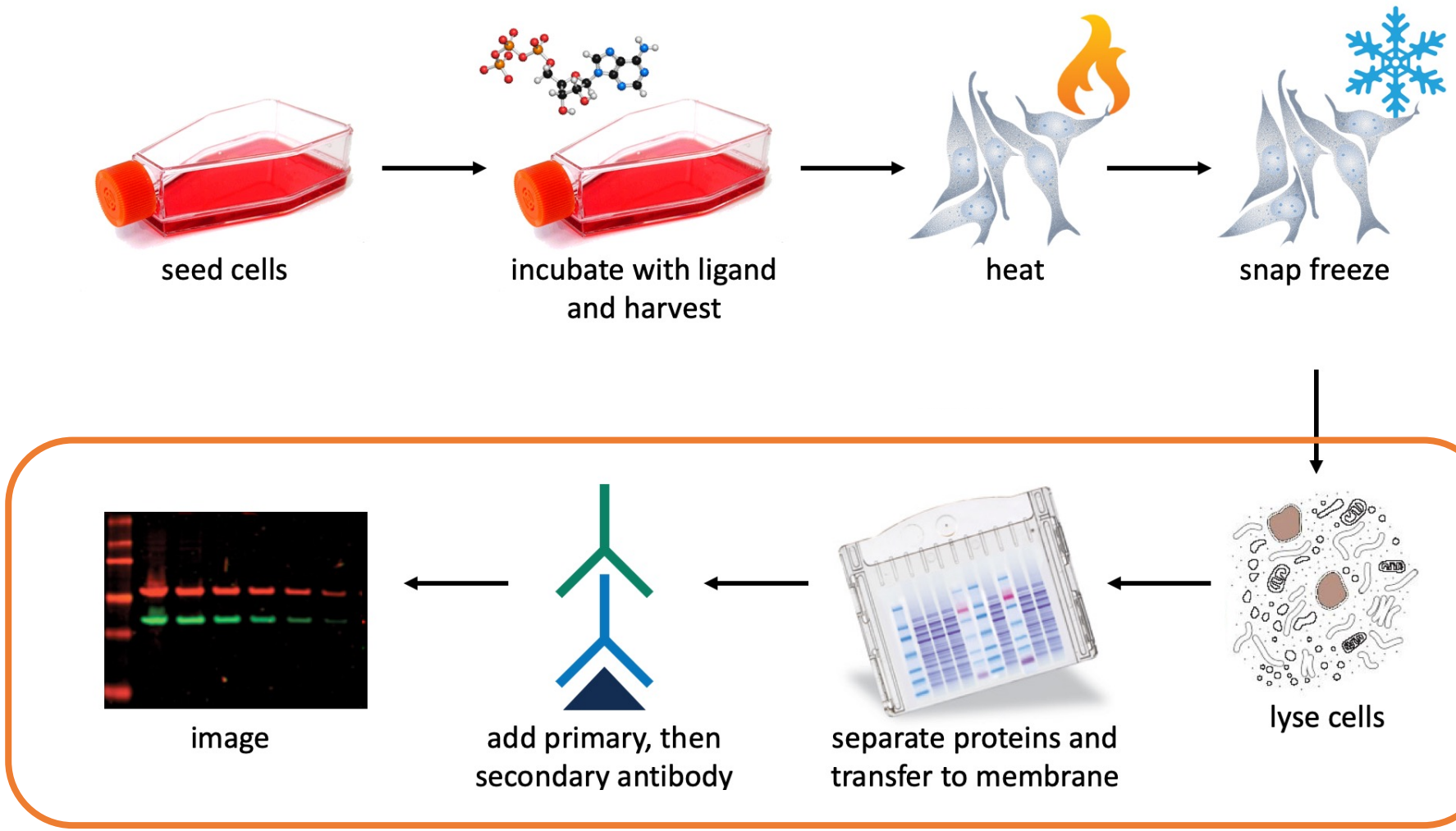


Treat Cells for CETSA

- **Heat:** Test protein stability and remove denatured proteins later
- **Snap freeze:** Multiple snap freeze / thaw cycles lyses cells

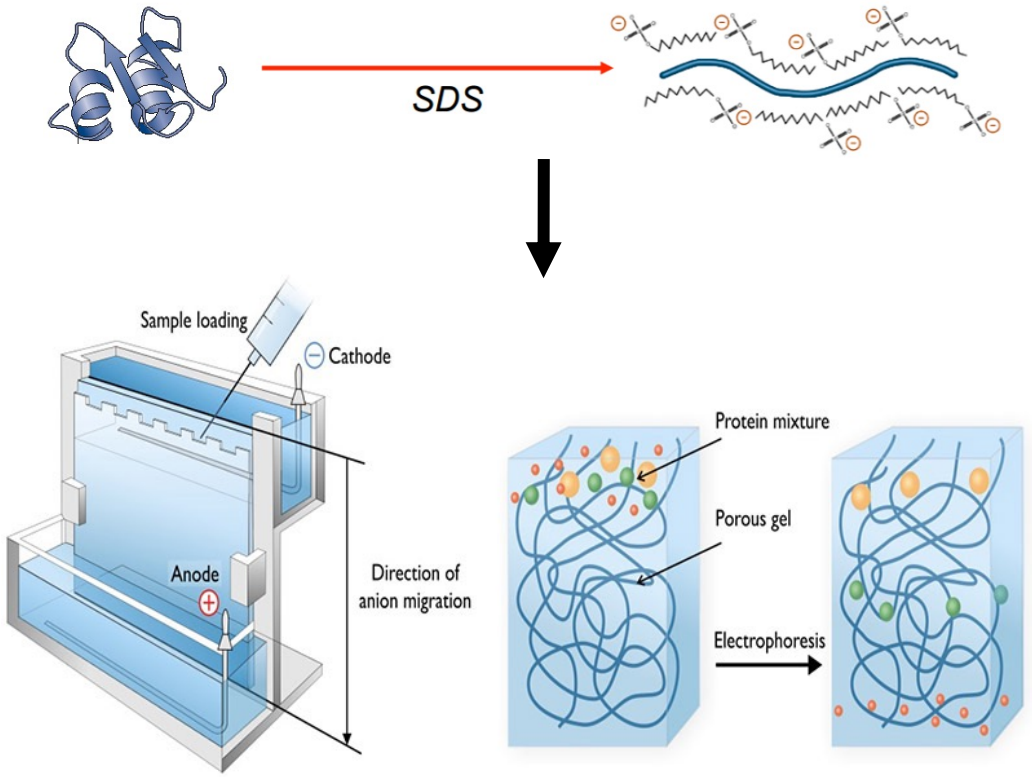


Imaging for CETSA



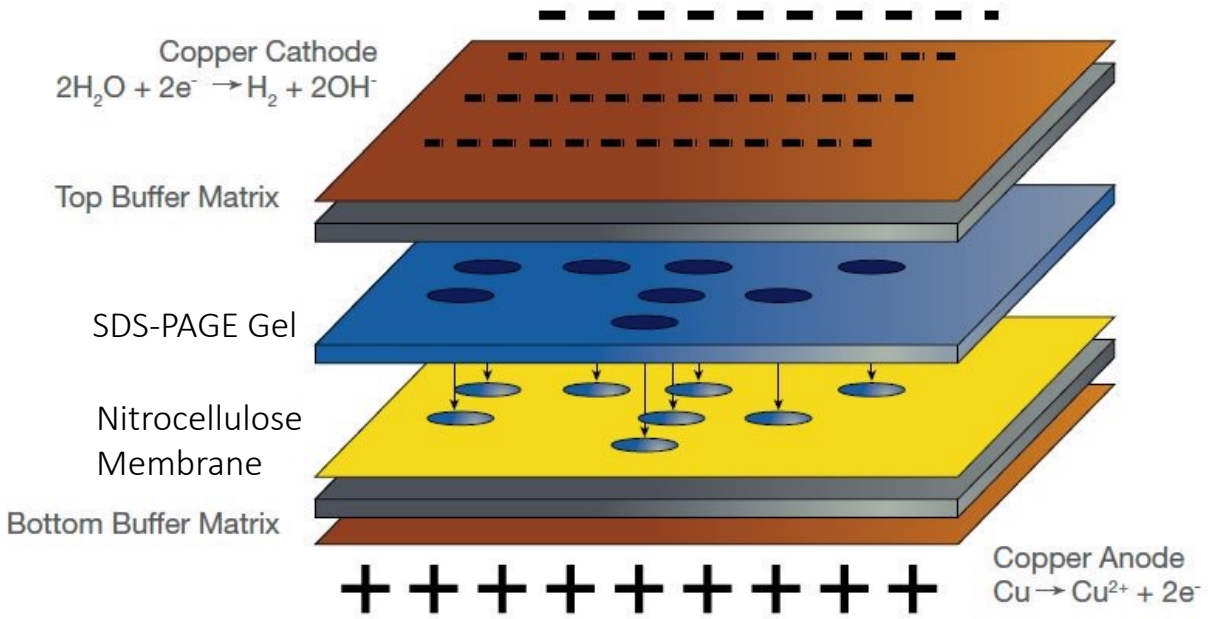
Separate proteins using SDS-PAGE

- SDS-PAGE separates proteins according to size
  - Charge and secondary structure are alleviated
- SDS imparts uniform charge-to-mass ratio



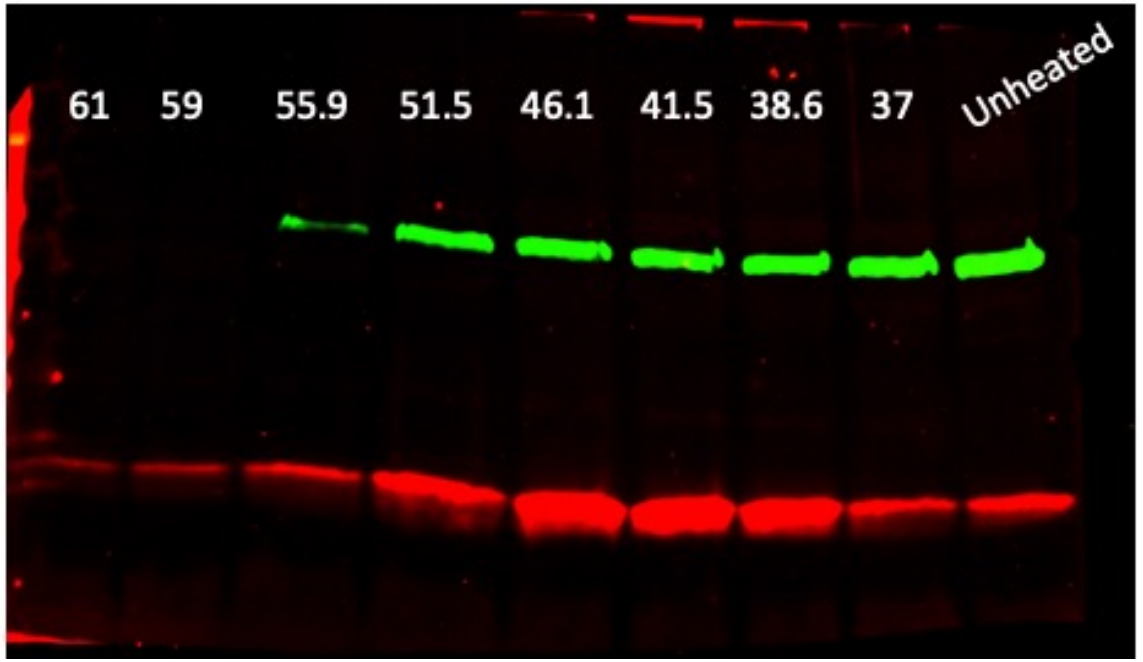
Transfer of proteins from gel to blot

- Allows for protein identification with detection antibodies
- Move protein bands from polyacrylamide gel to a nitrocellulose membrane for further assessment
- Net negatively charged proteins are migrated using a current from the gel to a membrane



Proteins are visualized through Western Blot

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



For today

- Work through wiki to do chose small molecule ligands and perform CETSA experiment

For M2D7 (4/22)…

- Write an outline of the research article discussion
  - Use citations
  - Propose 2 follow up experiments
- See wiki for additional guidance