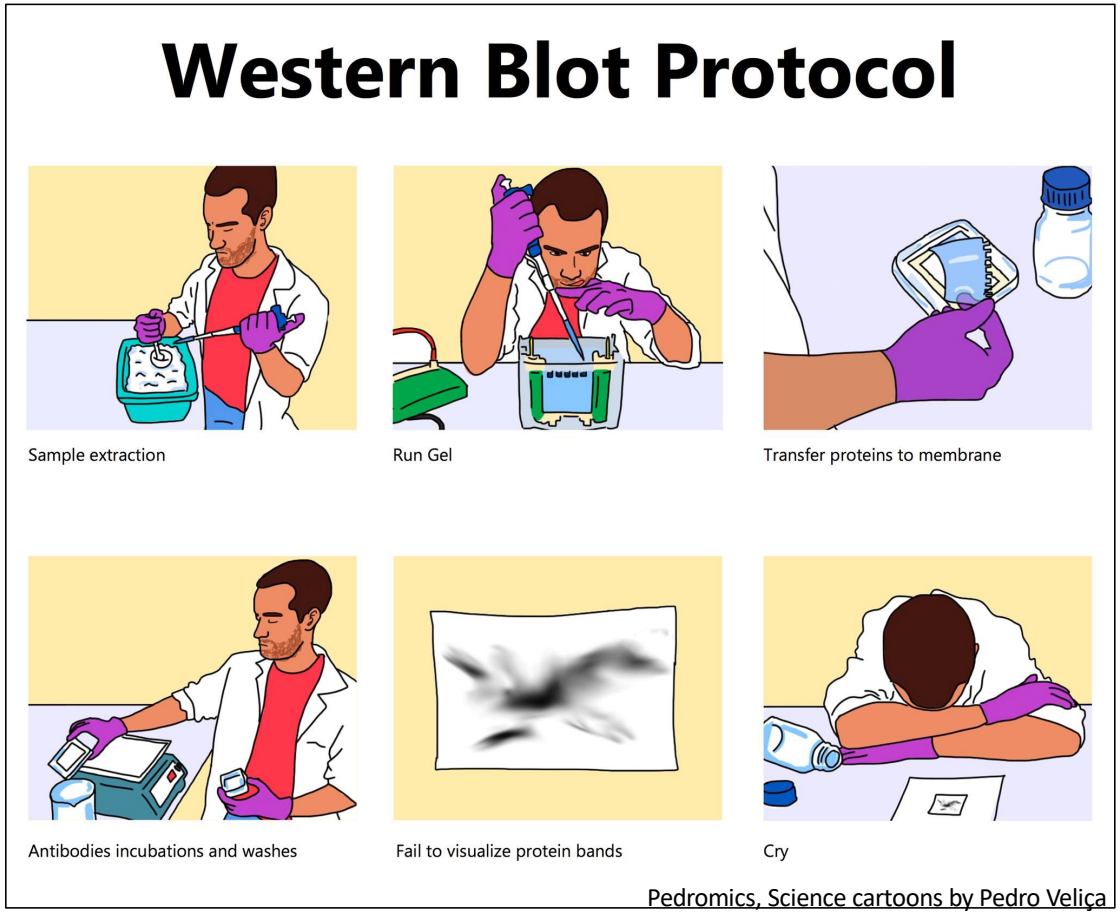
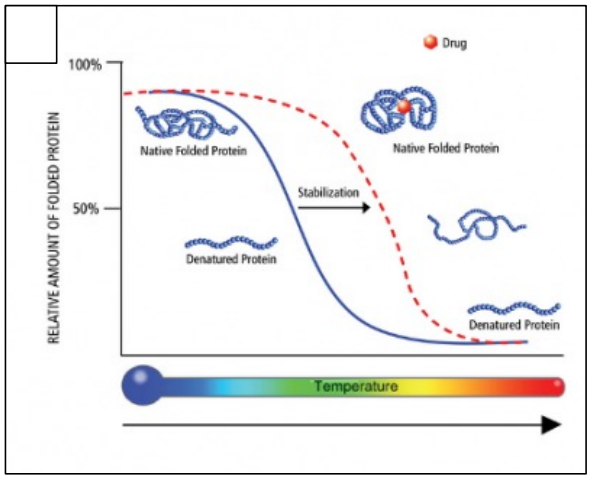
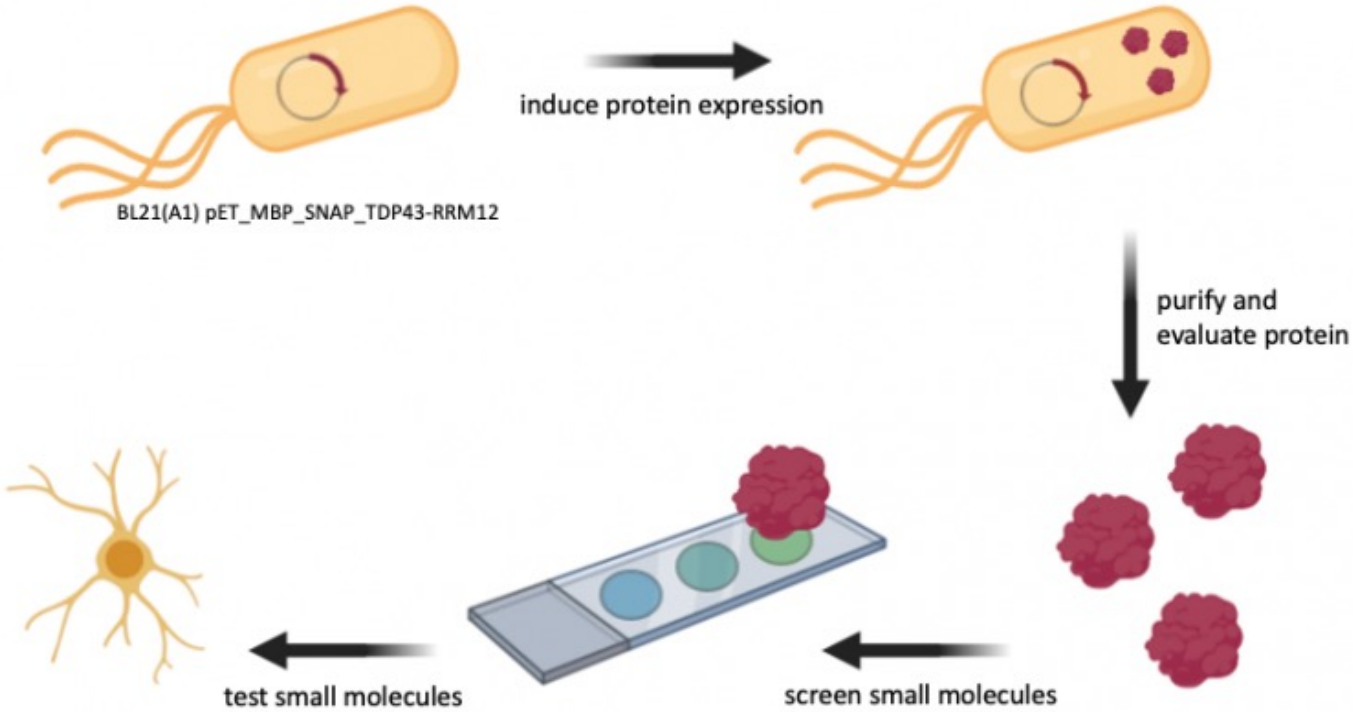
M2D6: Utilize cellular thermal shift assay (CETSA) to test putative small molecule binders

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)

ON THE MOD2 OVERVIEW PAGE:

PROTOCOL

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

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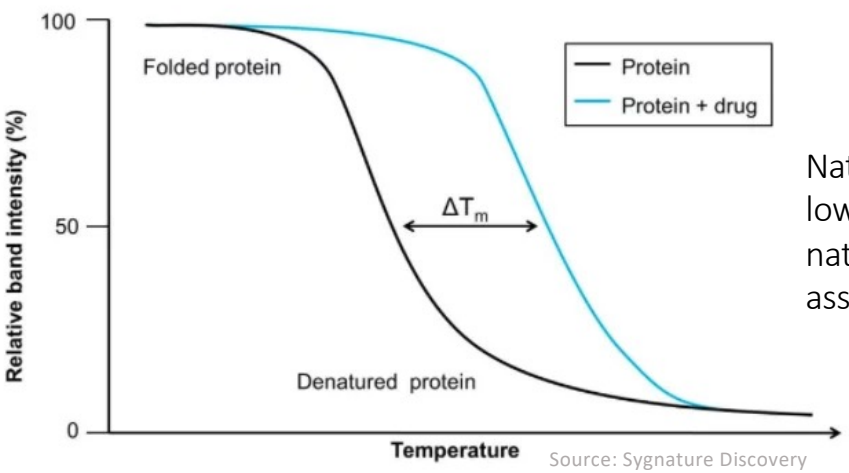

Source: Sygnature Discovery

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

- The ΔT_m indicates protein stabilization / destabilization compared to control
- Assesses thermal stabilization of protein in presence / absence of ligand **<u>in the cell</u>**

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, Maria Ignatushchenko, Tim Lundin, Thomas Hammarlund, Pär Nordlund[1] & Daniel Martinez Molina[1]

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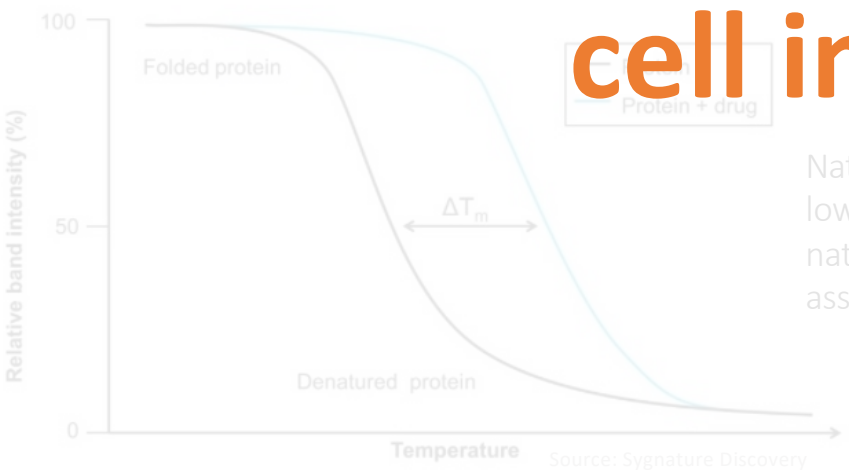
Why is testing for ligand binding in a cell important?

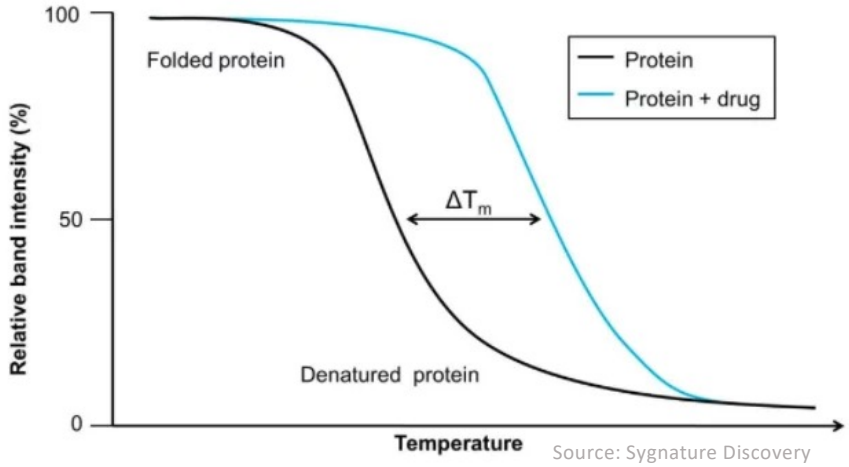
- The ΔT_m indicates protein stabilization / destabilization compared to control
- Assesses thermal stabilization of protein in presence / absence of ligand <u>in the cell</u>



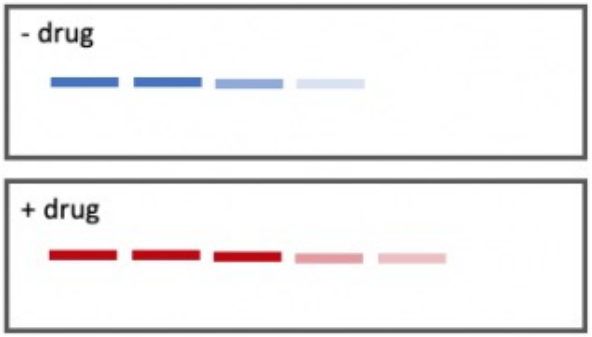
Source: Sygnature Discovery

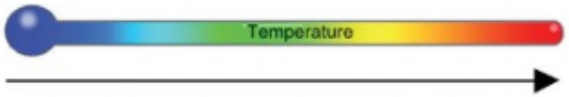
Native proteins denature at lower temperature than native folded proteins associated with drug

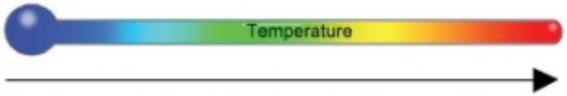
CETSA : Protein stability is measured by Western blot



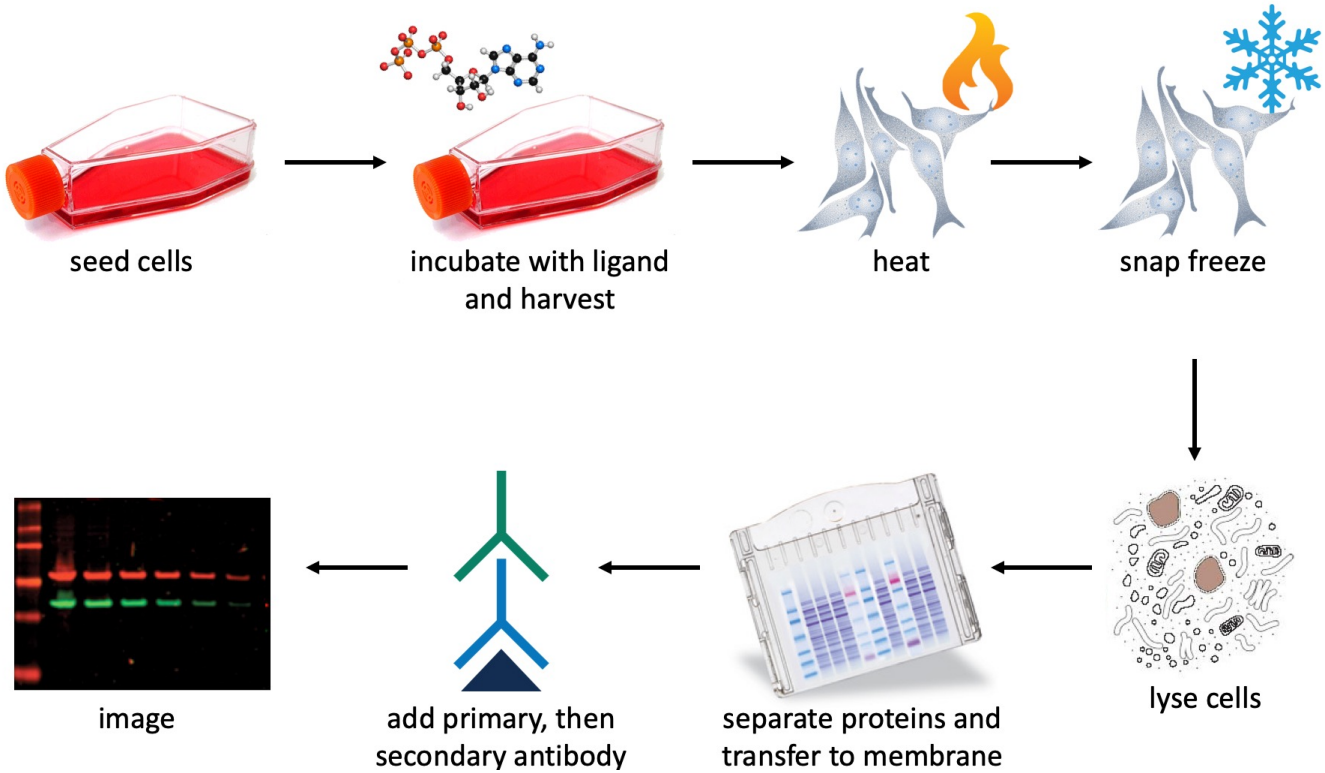
Identify presence of native folded proteins





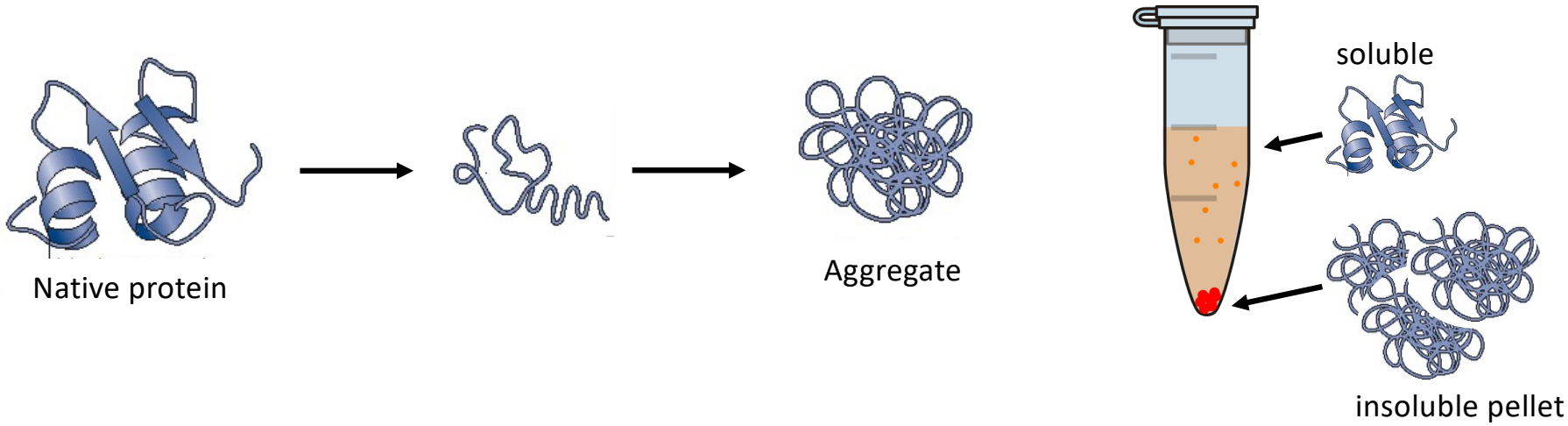


Major experimental steps of CETSA



Heat causes protein denaturation

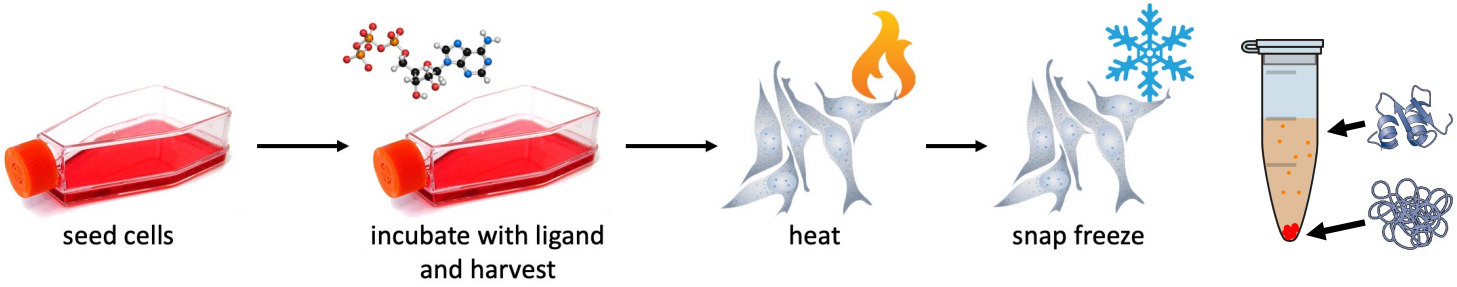
- As proteins denature, ‘melted’ primary structures aggregate
- Aggregates precipitate out of solution and can be removed via centrifugation



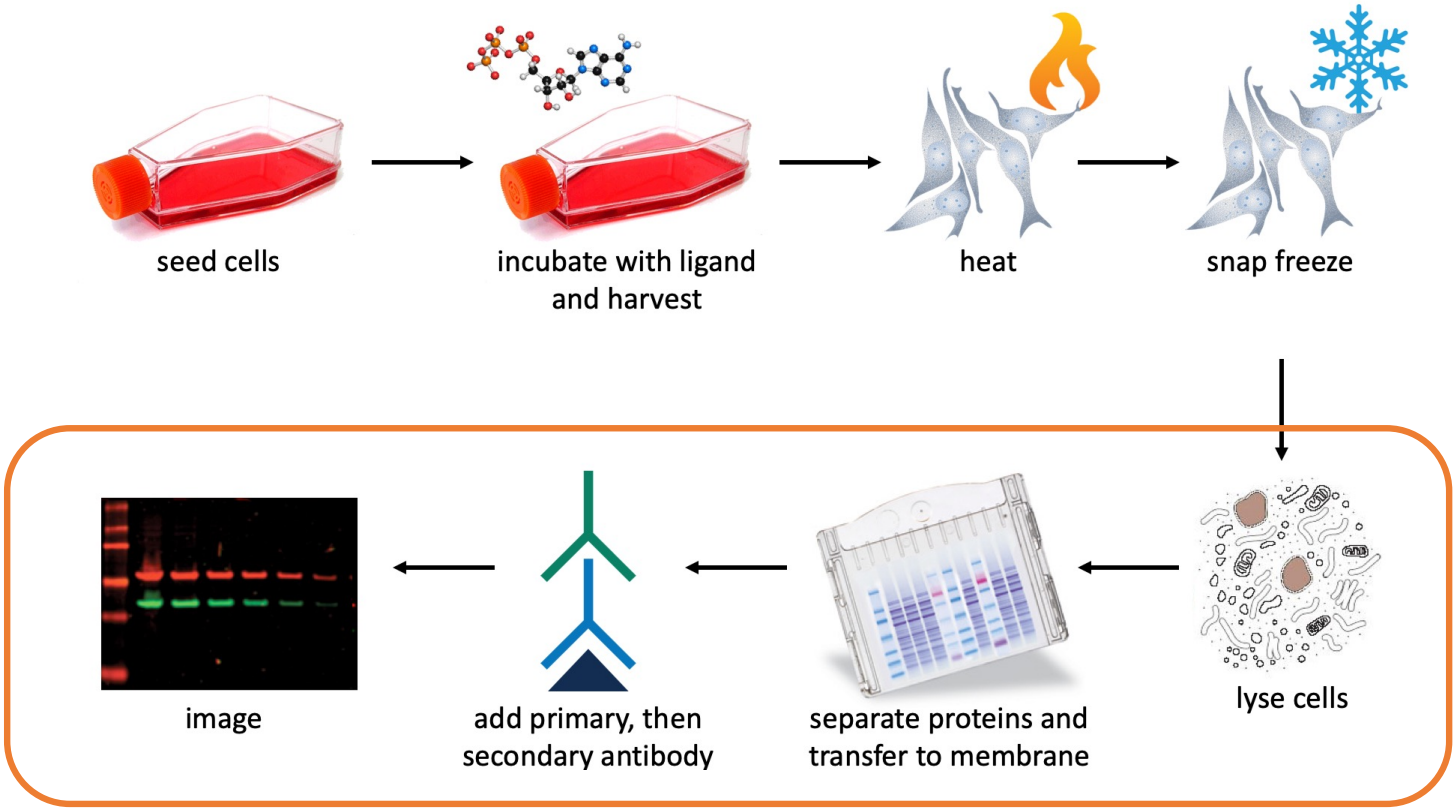
Treat Cells for CETSA

- **Heat:**

- **Snap freeze:**

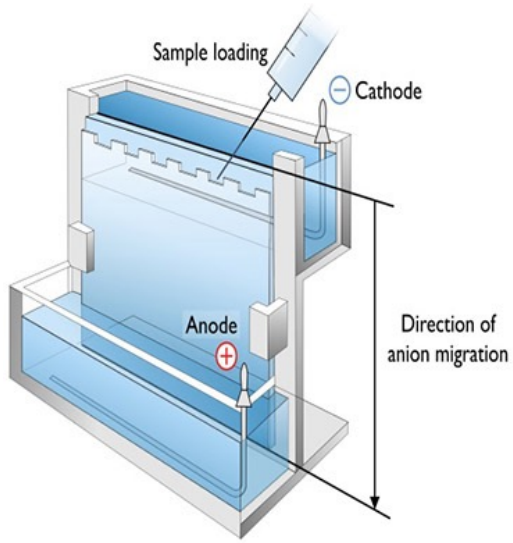


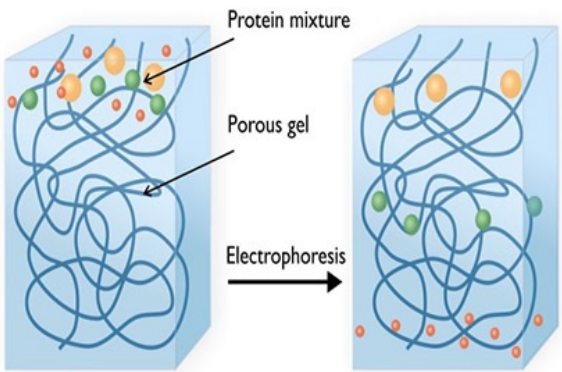
Imaging for CETSA



Separate proteins using SDS-PAGE (Review of M2D2 prelab)

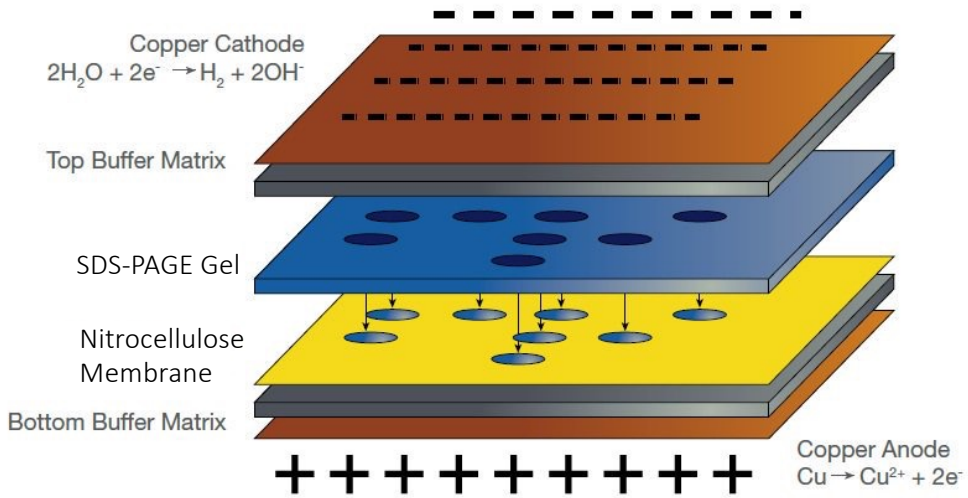
- SDS-PAGE separates proteins according to _____
  - Charge and secondary structure are alleviated by: _____
- SDS imparts uniform charge-to-mass ratio





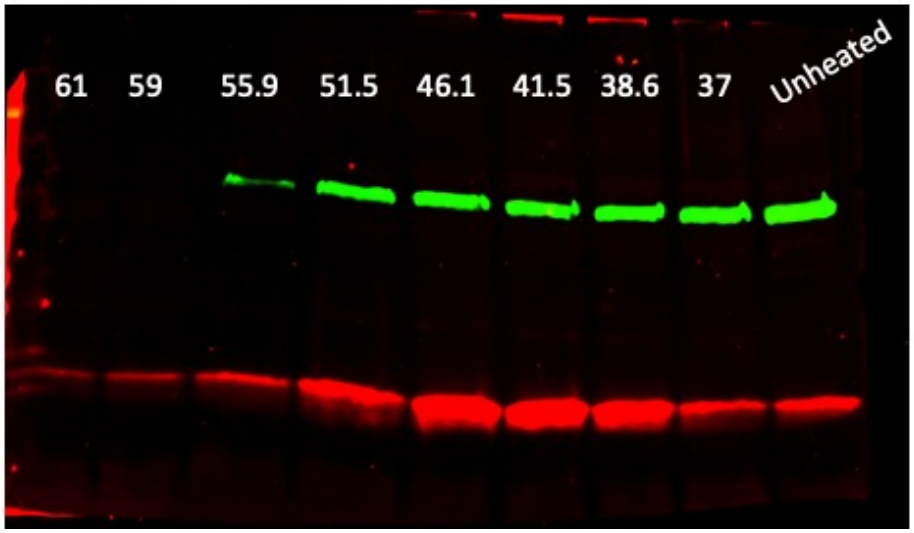
Transfer of proteins from SDS-PAGE gel to Nitrocellulose membrane

- 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



Tubulin

FKBP12

For today

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

For M2D7 (one week from today, 4/23)…

- Write an outline of the research article discussion
  - Use citations
  - Propose 2 follow up experiments

- See wiki for additional guidance