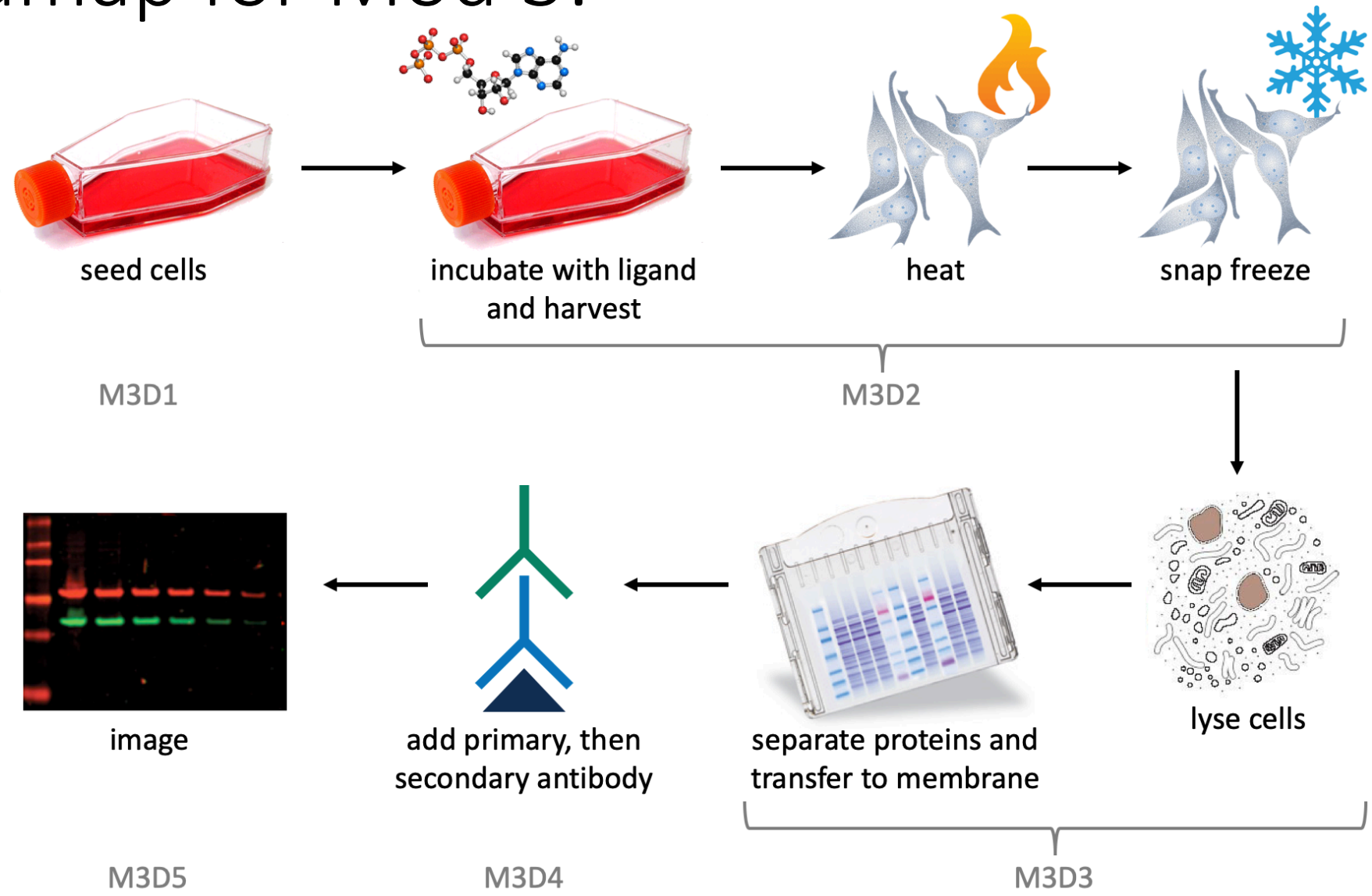
M3D2:
Incubate with ligand and apply heat treatment

1. Prelab discussion
2. Treat cells for CETSA

Important Mod 3 dates

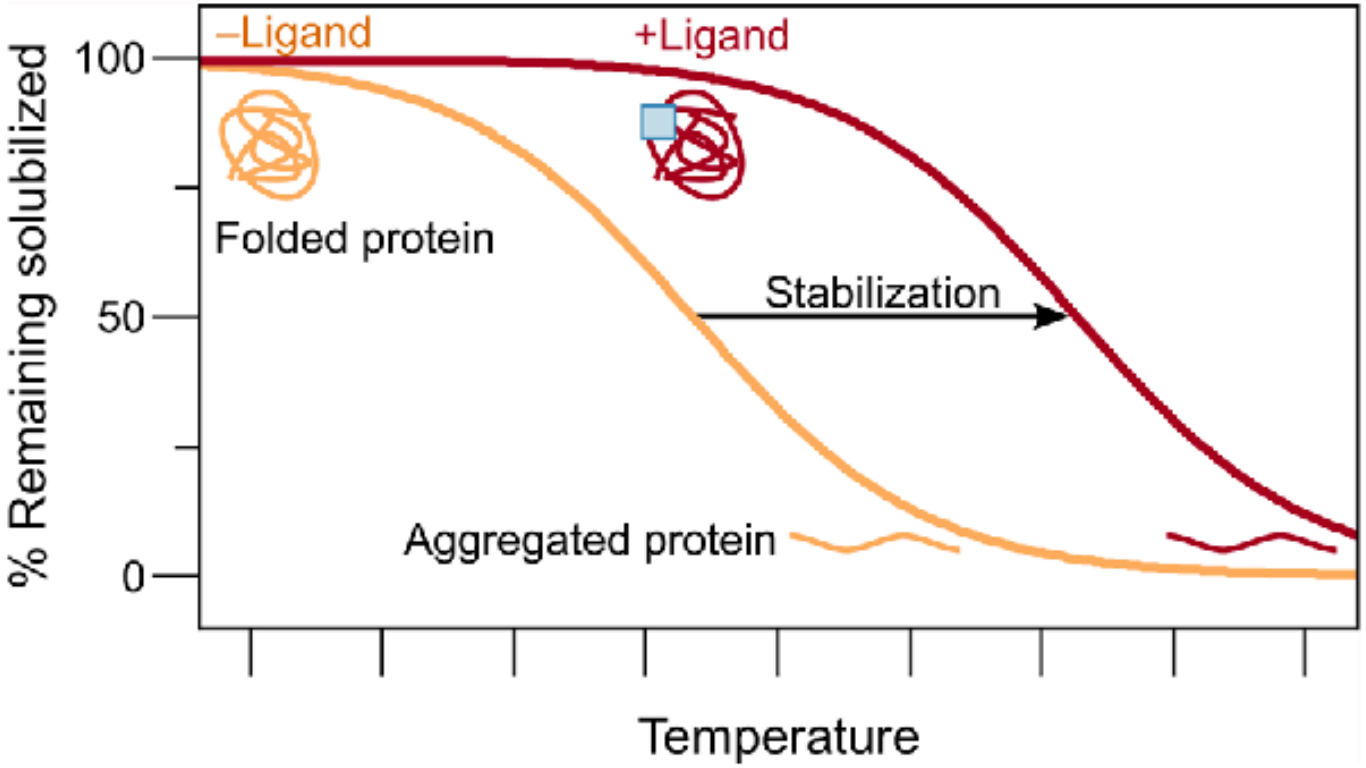
- Research proposal presentation due Thursday, Dec 5 by 1 pm
  - Completed in teams!
  - 12 minute presentation, submitted to Stellar
- Blog post due Friday, Dec 6 by 10 pm
- Mini-report due Monday, Dec 9 by 10 pm
  - Completed in teams!
  - 3 page word document, submitted to Stellar

Roadmap for Mod 3:



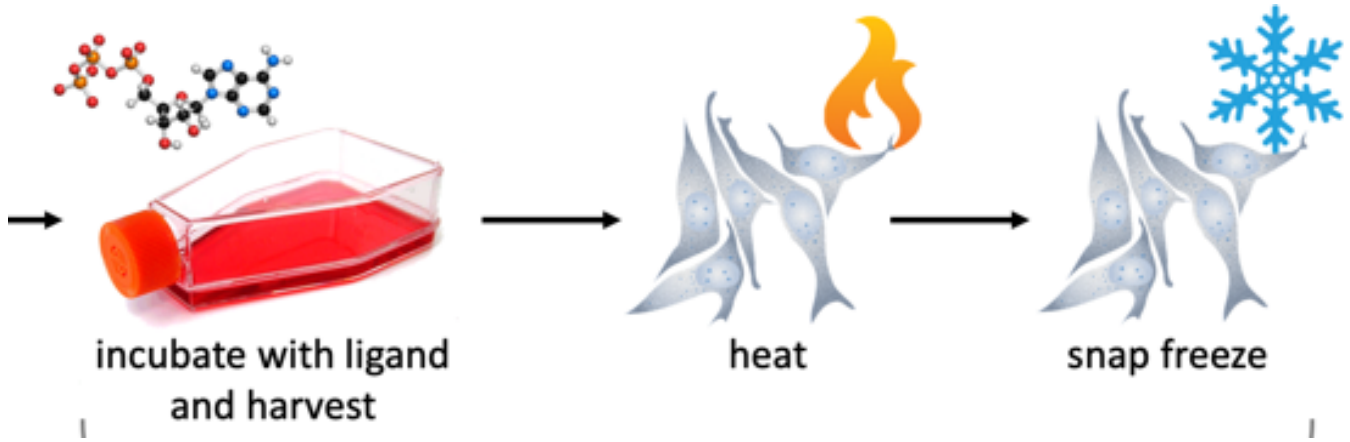
Cellular thermal shift assay (CETSA)

- Assesses thermal stabilization of protein in presence / absence of ligand
- As with DSF, the ΔT_m indicates protein stabilization / destabilization compared to control



CETSA cell preparation and ligand treatment

- Cells from previous laboratory session were used to seed 3 M cells for ligand treatment
- Today you will treat cells with ligand, harvest, heat, and snap freeze



Let’s take a closer look…

- Treat: ligand 3uM, 30uM experimental
  DMSO = neg. control
  rapamycin = pos control

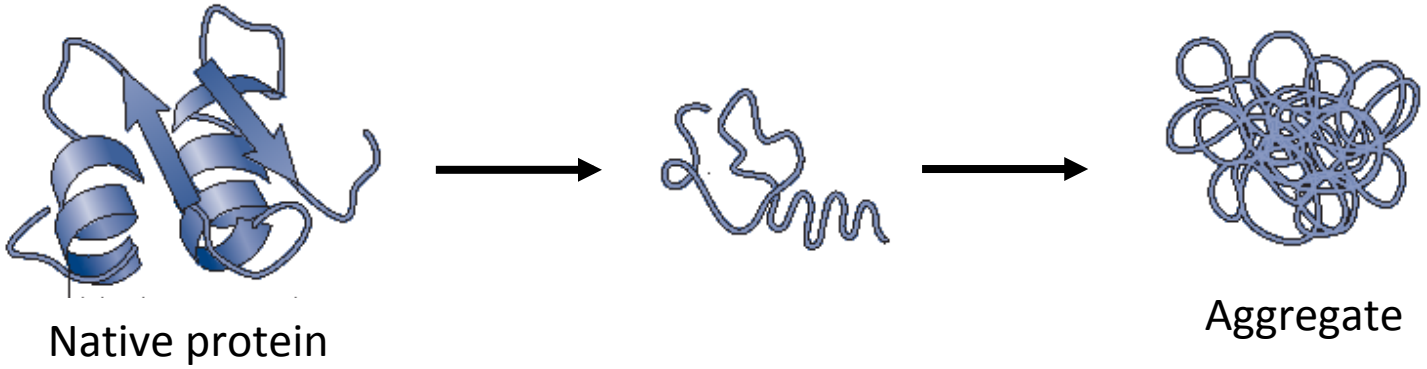
TC

- Harvest:
  collect cells
- Heat:
  59°C, 3 min
- Snap freeze:
  Storage, stops rxn

main lab

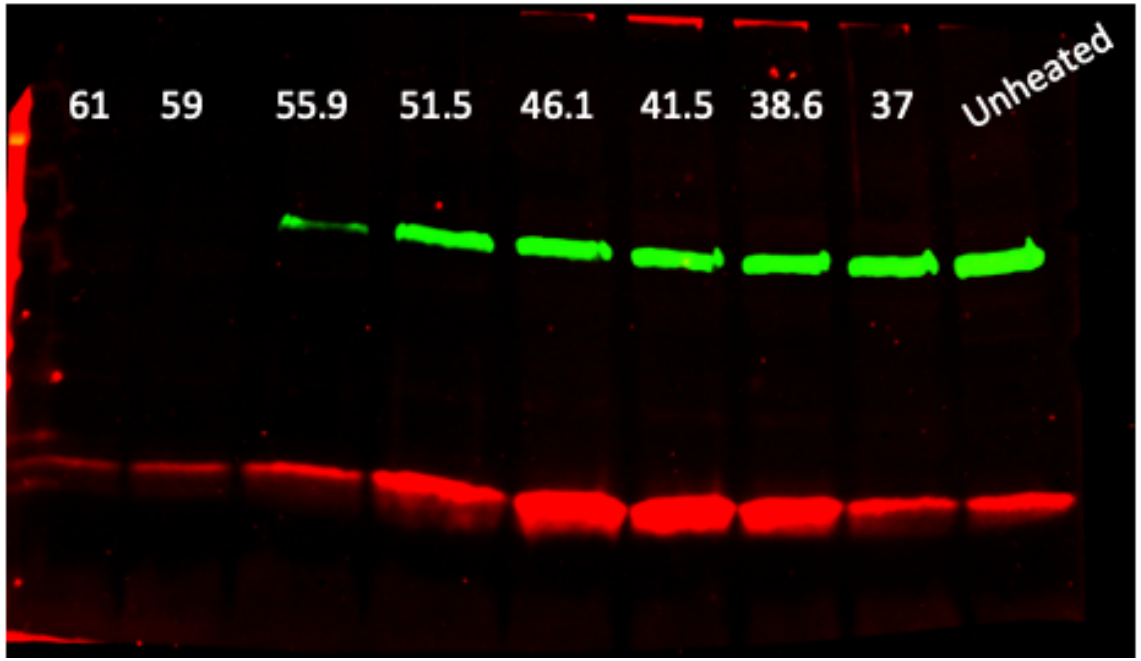
Heat causes protein denaturation

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



CETSA visualized via Western blot

- T_m determined by presence of protein bands following SDS polyacrylamide gel electrophoresis
- In pilot studies, 59 ℃ was identified as ideal temperature to assess FKBP12 in your experiment



For today…

- Please be patient as not everyone can be in the tissue culture room at the same time!

For M3D3…

- With your laboratory partner, write a paragraph concerning the research question you would like to pursue for your research proposal
  - See prompts on wiki
  - Does not need to be your finalized research idea / project