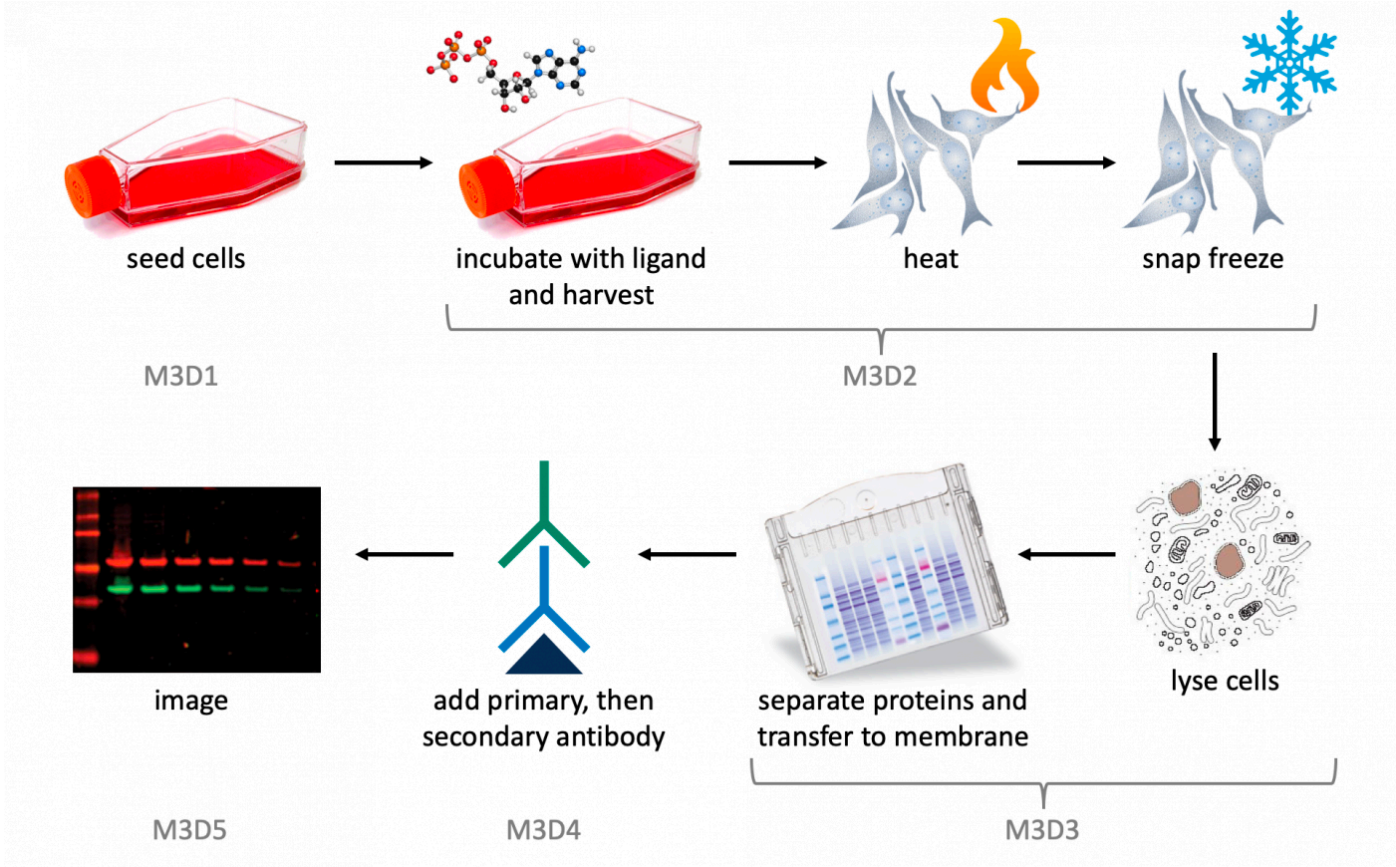
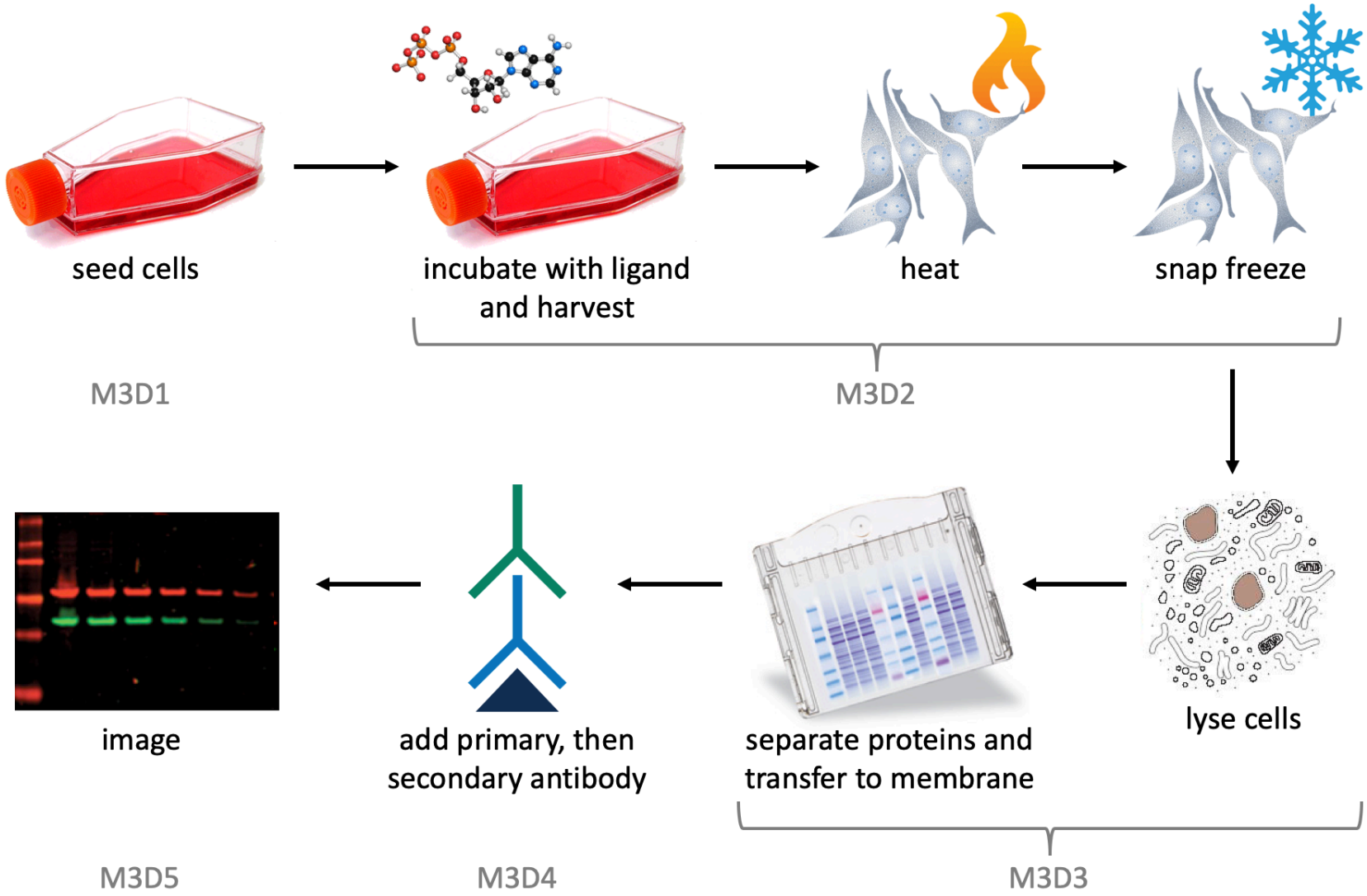
M3D2 Pre-Lab: Incubate with ligand and apply heat treatment for protein denaturation



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

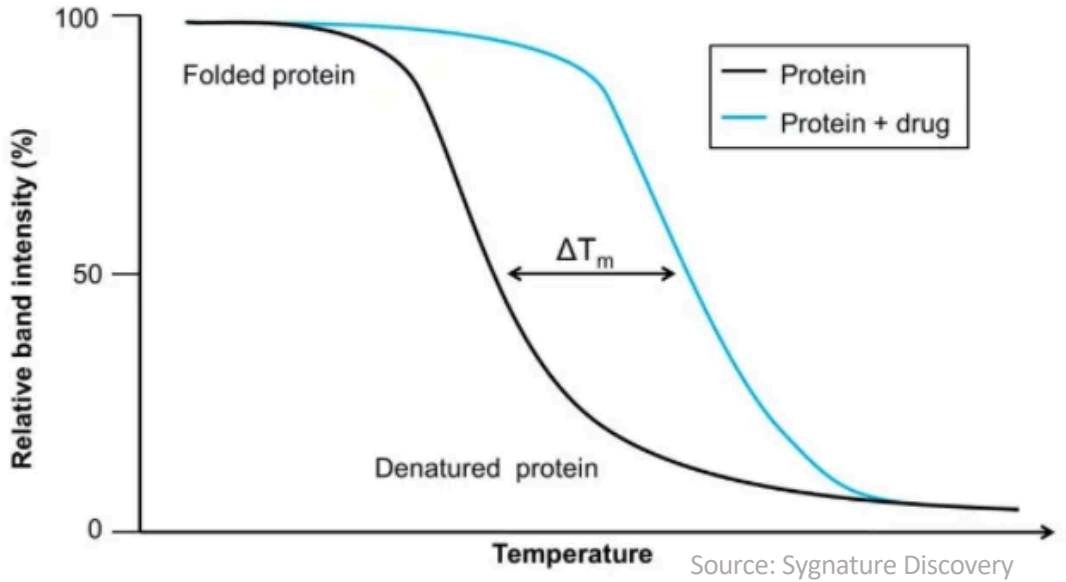
Roadmap for Mod 3



Today

1. Prepare media for CETSA experiments
2. Incubate cells with ligand and harvest for CETSA

Cellular thermal shift assay (CETSA)



Source: Sygnature Discovery

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

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]

[1]Department of Medical Biochemistry and Biophysics, Division of Biophysics, Karolinska Institutet, Stockholm, Sweden. [2]Chemical Biology Consortium Sweden, Science for Life Laboratory Stockholm, Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Solna, Sweden. Correspondence should be addressed to T.L. (thomas.lundback@ki.se), P.N. (par.nordlund@ki.se) or D.M.M. (daniel.martinez.molina@ki.se).

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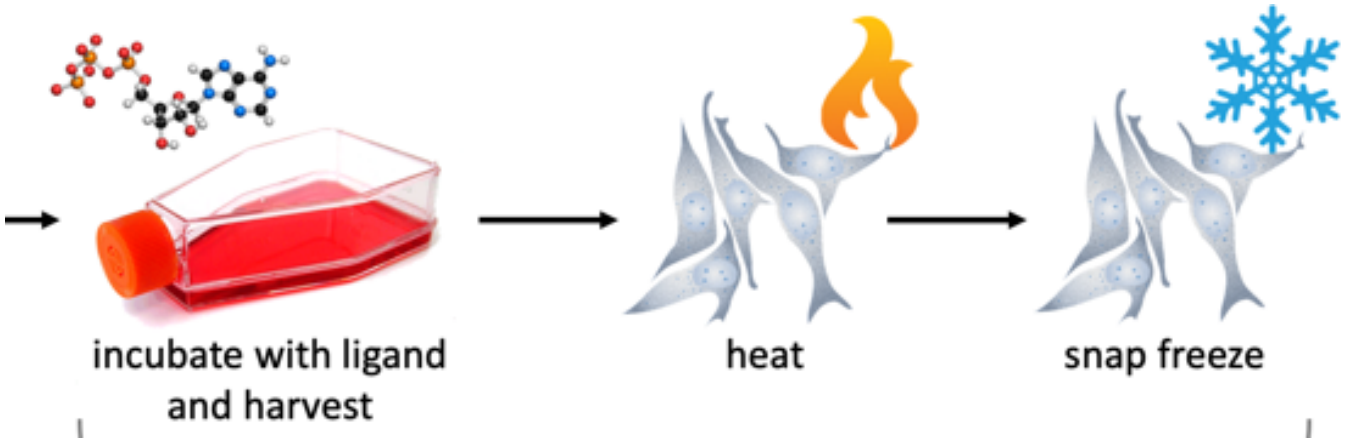
CETSA cell preparation and ligand treatment

- 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

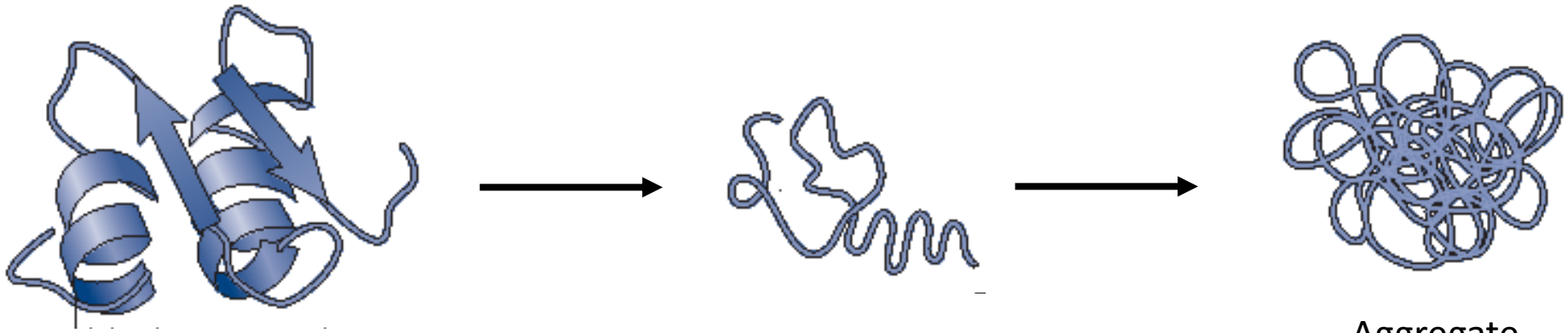


Taking a closer look…

- Treat: Ligand 14 → 3μM, 30μM] experimental
  DMSO — — controls
  rapamycin – +
- Harvest: spinning & collecting cells
- Heat: 59°C for 3 minutes
- Snap freeze: stop rxn & store

Heat causes protein denaturation

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

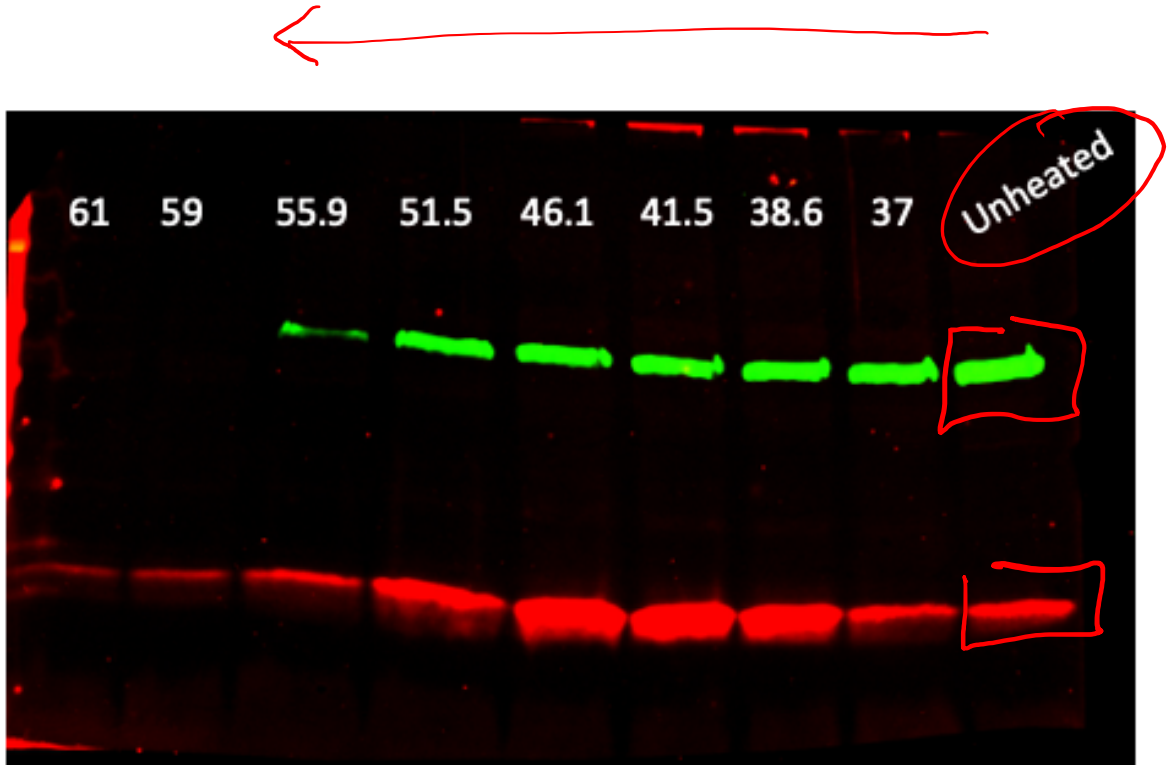


Native protein

Aggregate

CETSA visualized via Western blot

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



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