M3D1:
Prepare for cellular thermal shift assay (CETSA)

1. BE Comm Lab workshop
2. Prelab discussion
3. Expand cells for CETSA
4. Select ligand for CETSA

You will build upon work of previous 109ers!

- Research goal: Test ligands identified as putative binders of FKBP12 using *in-vivo* whole cell assay
  - Ligands were identified using small-molecule microarray (SMM)
  - *In-vitro* assays were used for initial tests: Peptidyl-Prolyl Isomerase (PPIase) assay and Differential Scanning Fluorimetry (DSF)

- Experimental approach: Use the Cellular Thermal Shift Assay (CETSA) to measure protein stability in response to ligand

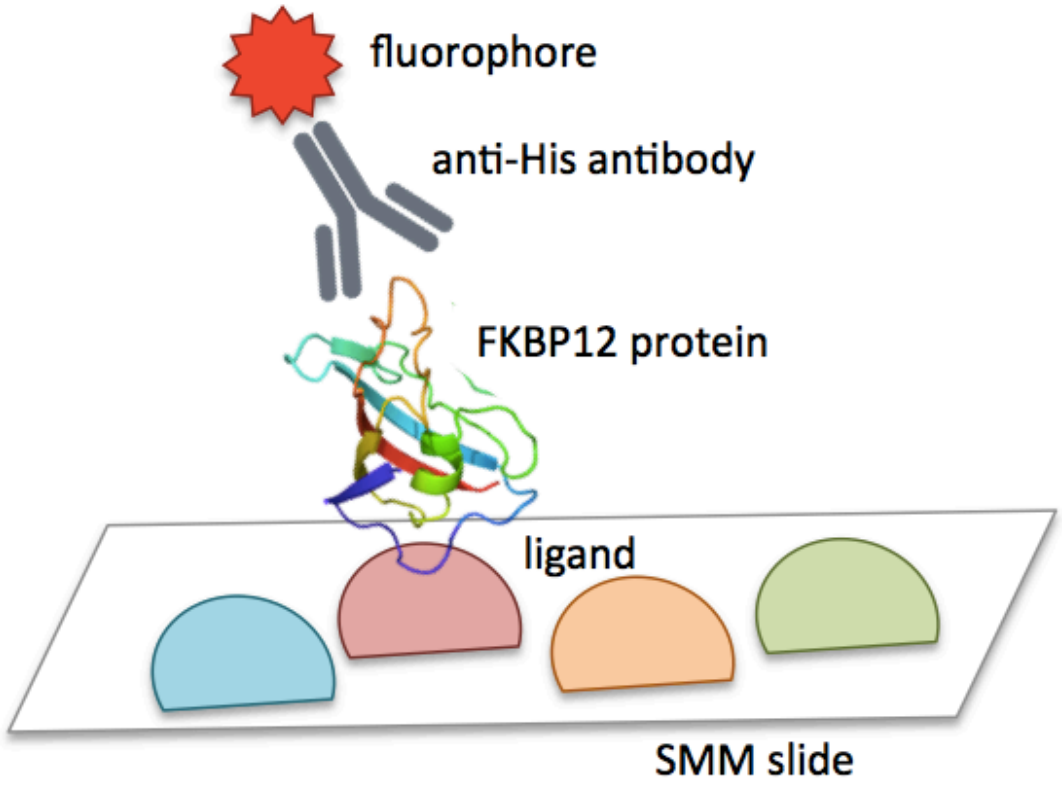
FKBP12 functions as a chaperone protein and is involved in immunosuppression

- Facilitates proper folding in proteins that contain proline residues

- Binds immunosuppressant molecule important in treating autoimmune disorders and preventing organ transplant rejection
  - Inhibits phosphorylation step thereby blocking immune response

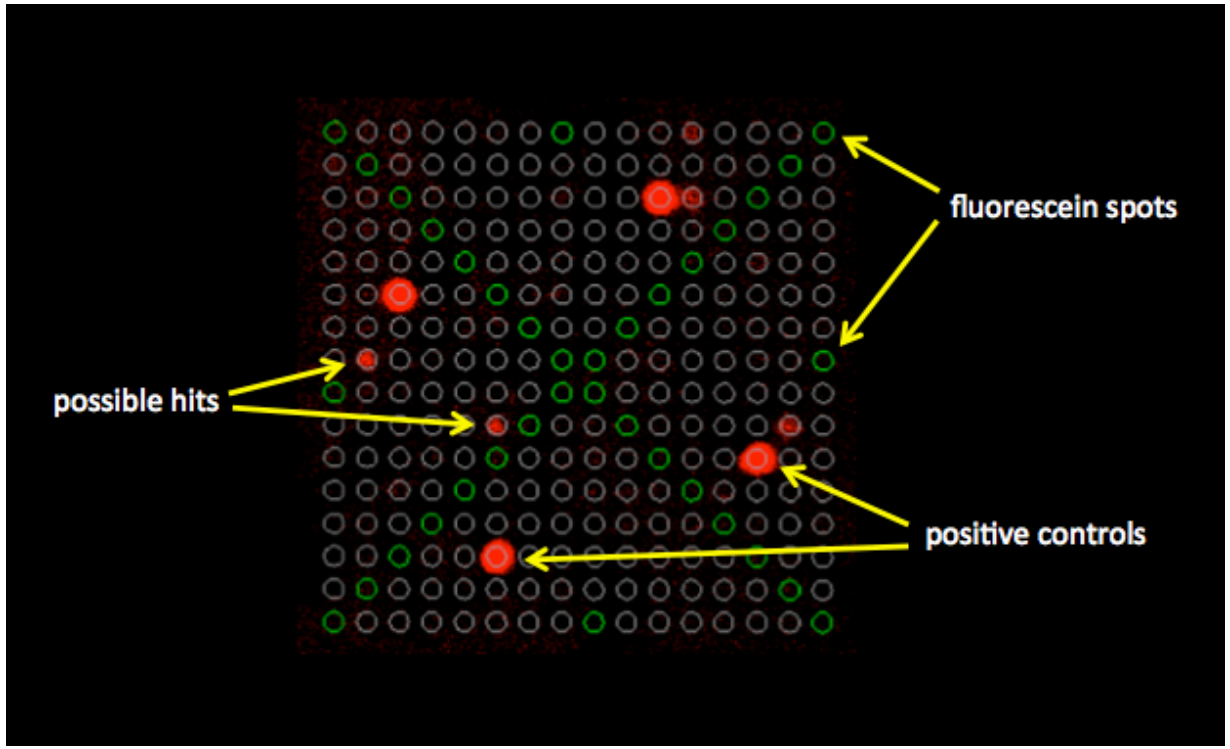
Ligands were identified using SMM

- Slides are printed with small molecules, or ligands
- Protein of interest is washed over the slide
  - If protein is able to bind ligand, will remain on slide at the specific location of the printed ligand
- Fluorescently labeled antibody is used to visualize location of bound protein / identify ligand



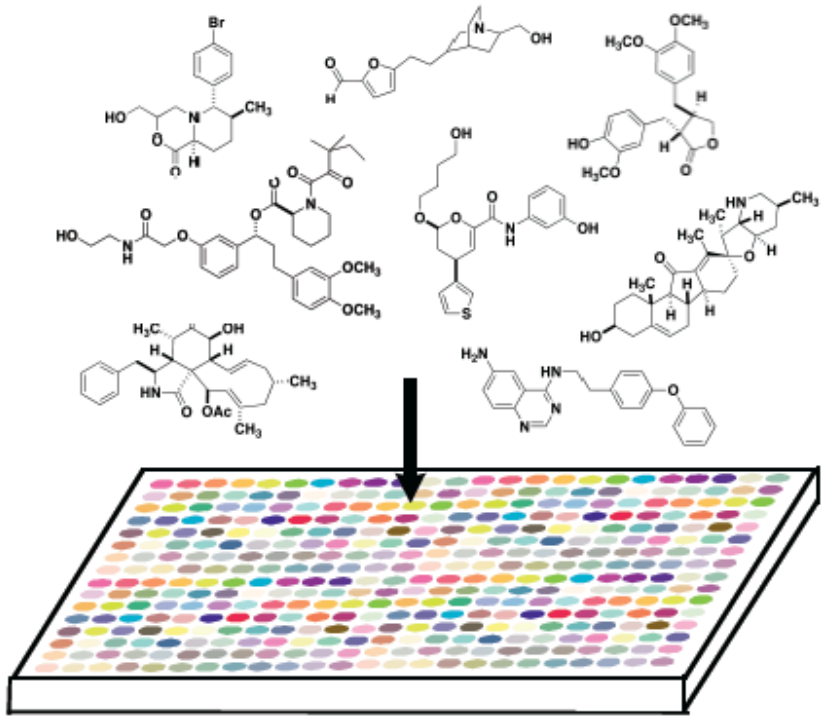
Positive hits will show as fluorescent spots

- Sentinel (fluorescein) spots used to align ‘hits’ such that the putative ligand binder is easily identified via the location of the spot on the slide
- Positive controls included to ensure assay was successful



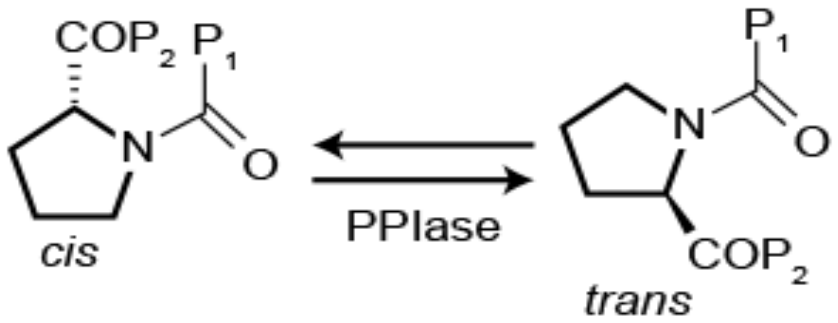
Results of SMM screen

- A total of 12,288 ligands examined
  - 2 replicate slides
  - 4 replicate spots for each ligand
- 16 ligands identified as putative binders
  - Based on z-score analysis and visual inspection of spots
  - Promiscuous binders omitted



In-vitro assays were used for initial tests: PPIase

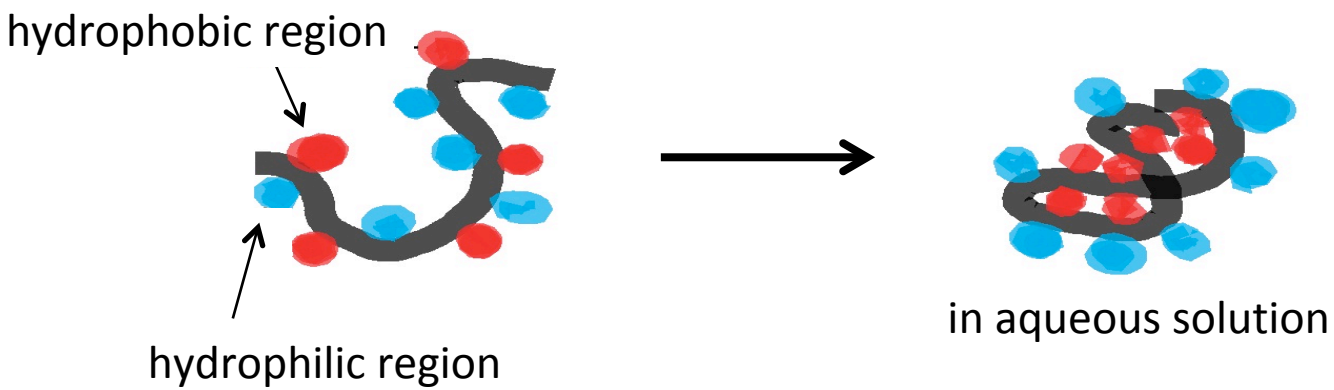
- FKBP12 has peptidyl prolyl cis-trans isomerase (PPIase) activity



- Important in role as protein folding chaperone at proline residues
- PPIase assay used to confirm FKBP12 activity for DSF assay

In-vitro assays were used for initial tests: DSF

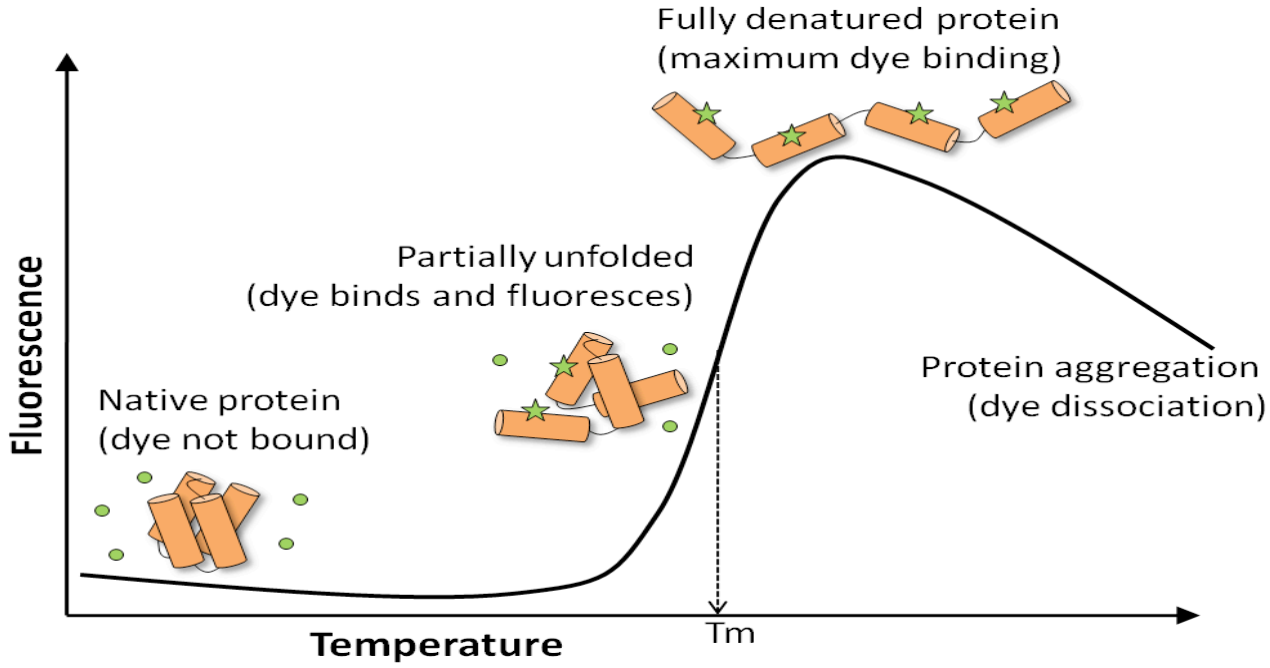
- Able to probe protein folding by adding a dye that interacts with hydrophobic regions of proteins
  - If protein is folded, dye is unable to access hydrophobic residues and is inactive (fluorescence quenched in aqueous solution)
  - As protein unfolds, dye binds hydrophobic residues and emits fluorescent signal



Proteins destabilize at high temperatures

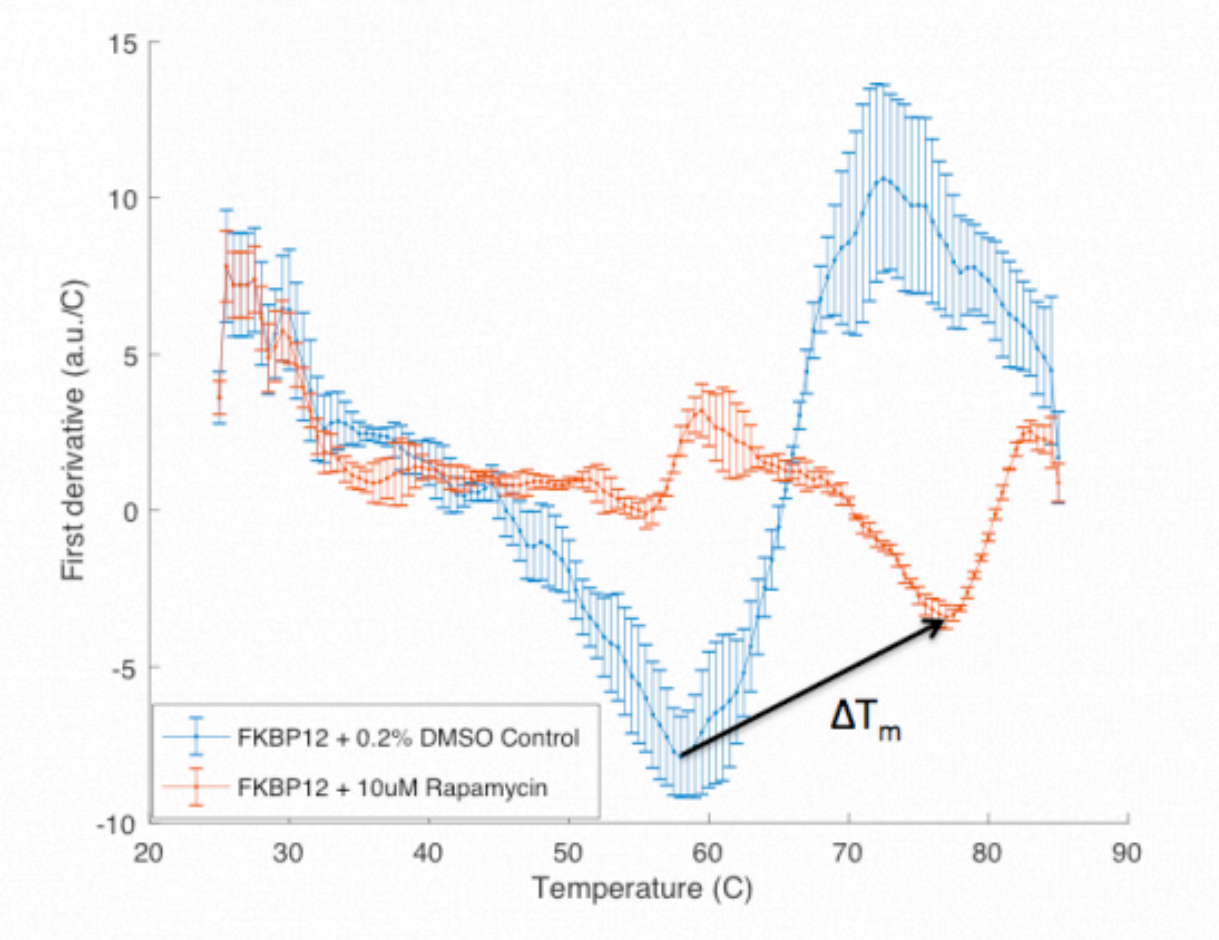
- Differential scanning fluorimetry (DSF) used to test protein stability (folding / unfolding) in response to added ligand as temperature increases

unbound (inactive) dye
bound (active) dye



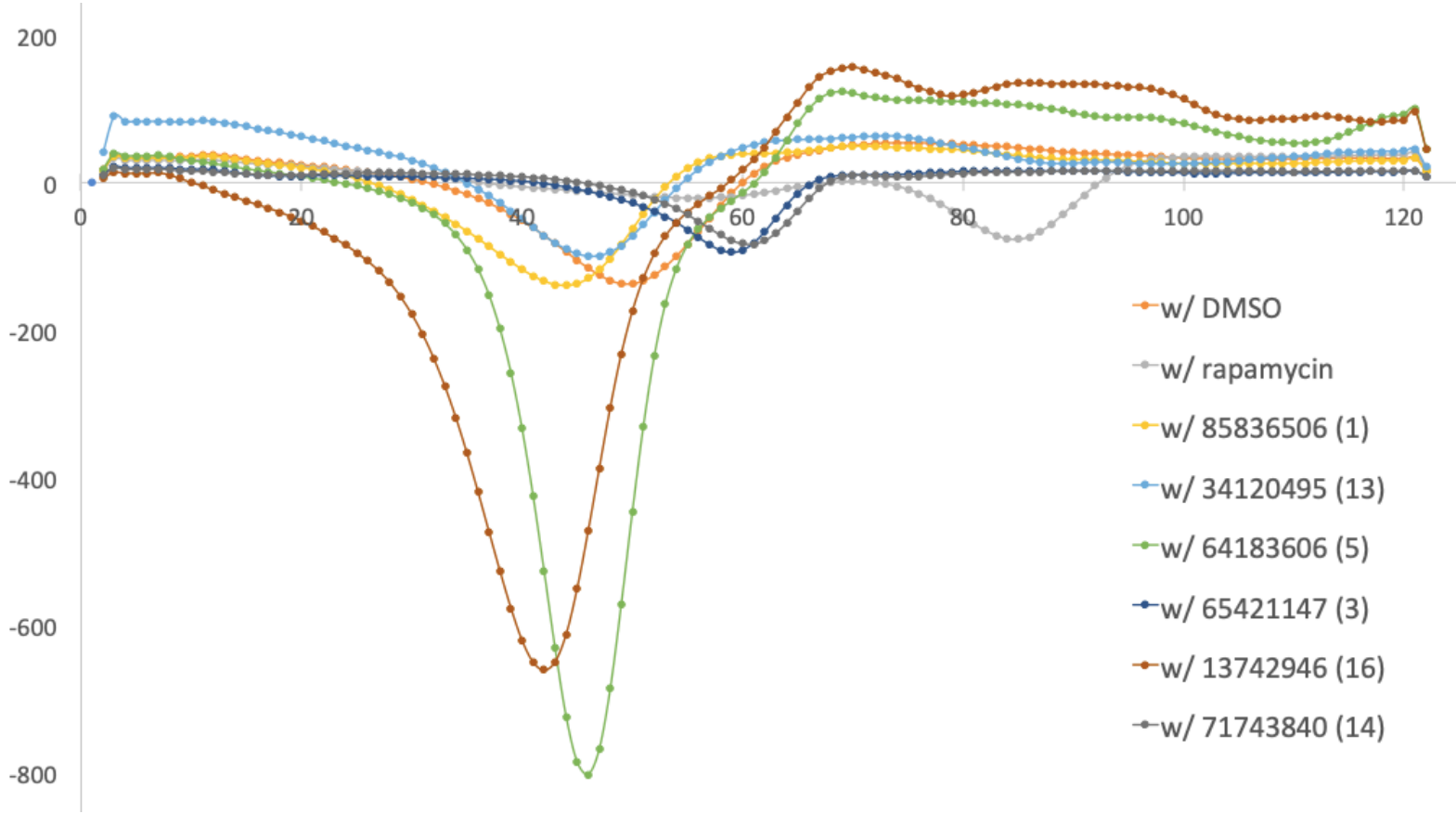
How are DSF data represented?

- T_m represents inflection point at which 50% of the protein is unfolded
- Shift in T_m (ΔT_m) indicates that the ligand altered the stability of the protein

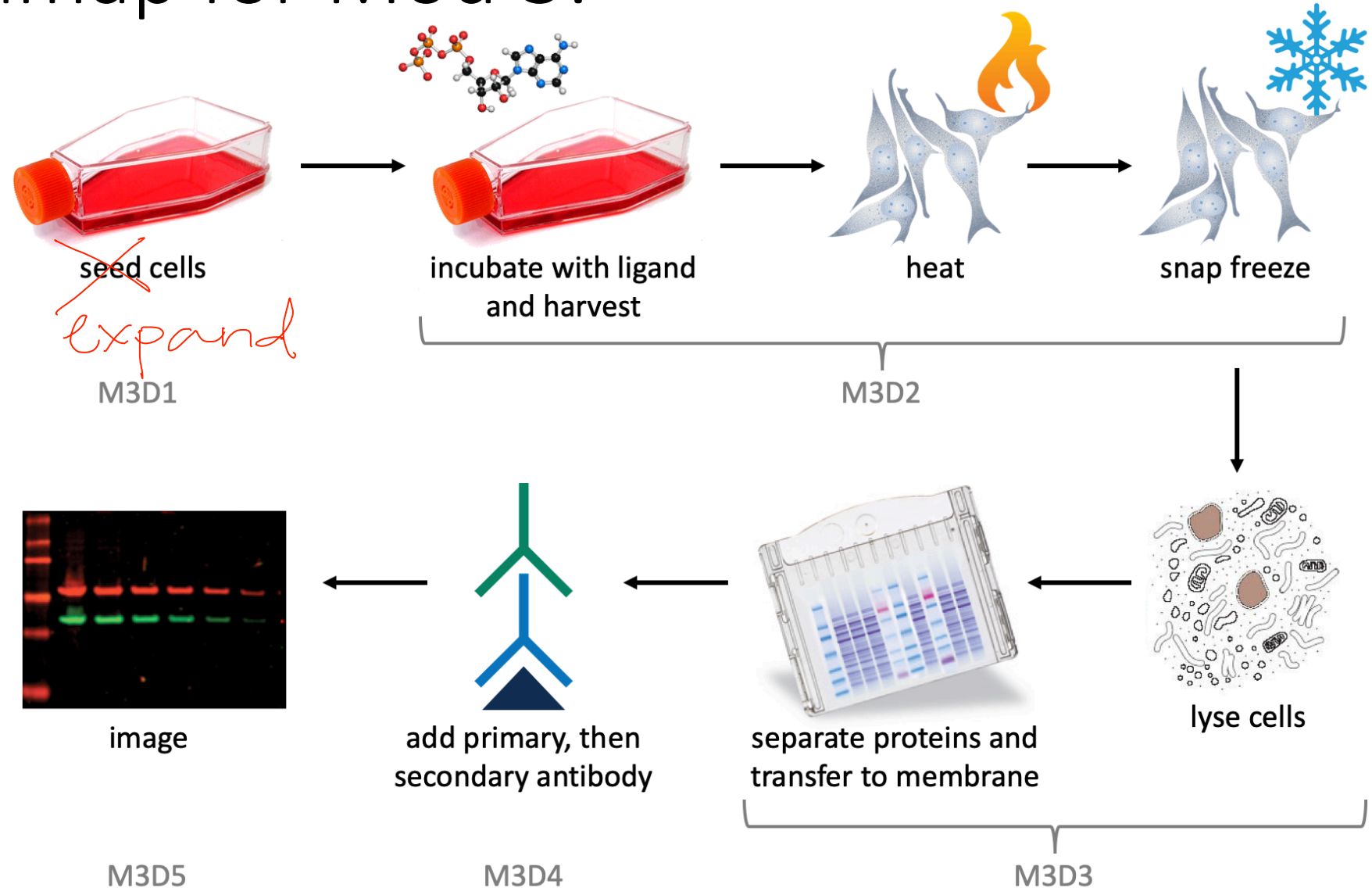


Of 16 tested ligands, 6 showed interesting DSF result

- Each team will choose one ligand to test using *in-vivo* cellular assay

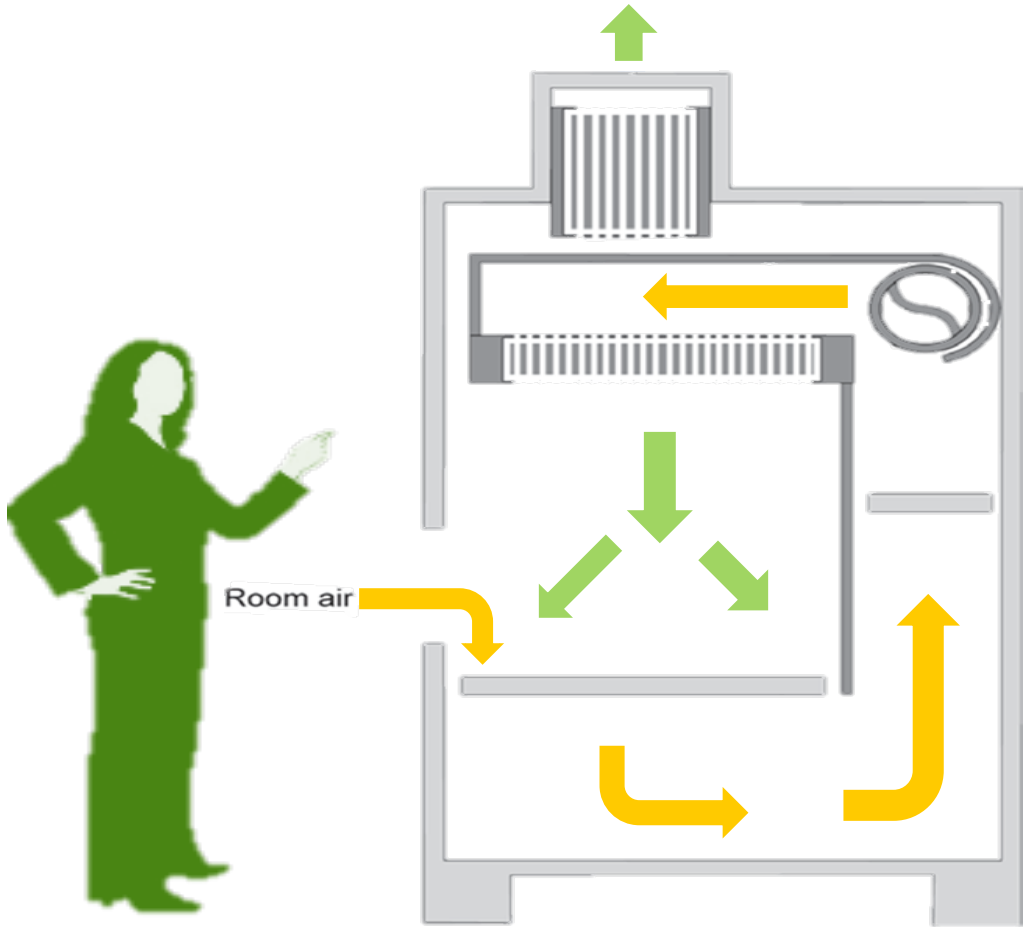


Roadmap for Mod 3:



Remember your cell culture best practices

- 70% ethanol everything:
  - Wipe cabinet before and after use
  - Wipe everything that enters the cabinet
  - Do not spray cells with EtOH
- Do not disturb air flow:
  - Do not block grille or slots
  - Minimize side-to-side arm movements
  - Work > 6” away from sash
  - Leave blower *on always*
- Do not open bottles / sterile supply containers outside!



For today…

- Remember to select your ligand on the sheet at the front laboratory bench!

For M3D2…

- Search the literature and describe FIVE recent articles that you find interesting. Consider how the findings can be developed for your Research Proposal presentation!
  - Include citation information for each article
  - Write 3-5 sentences that summarize the key finding