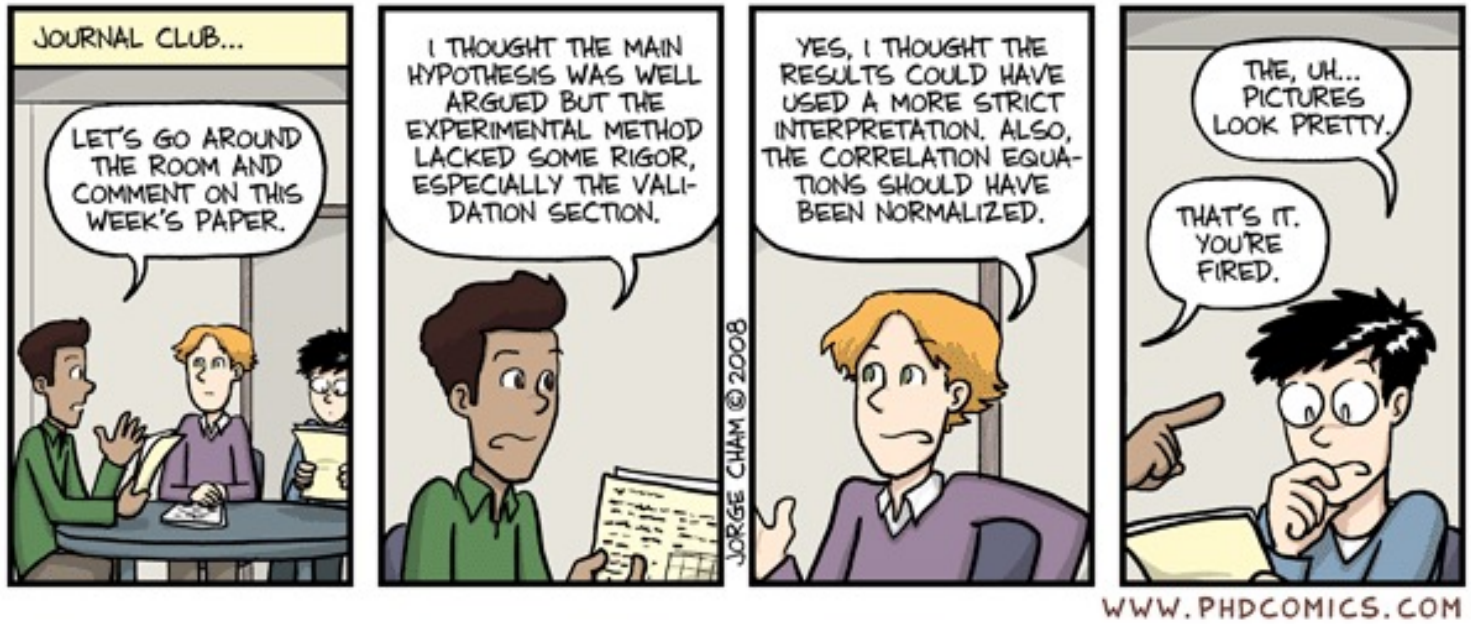
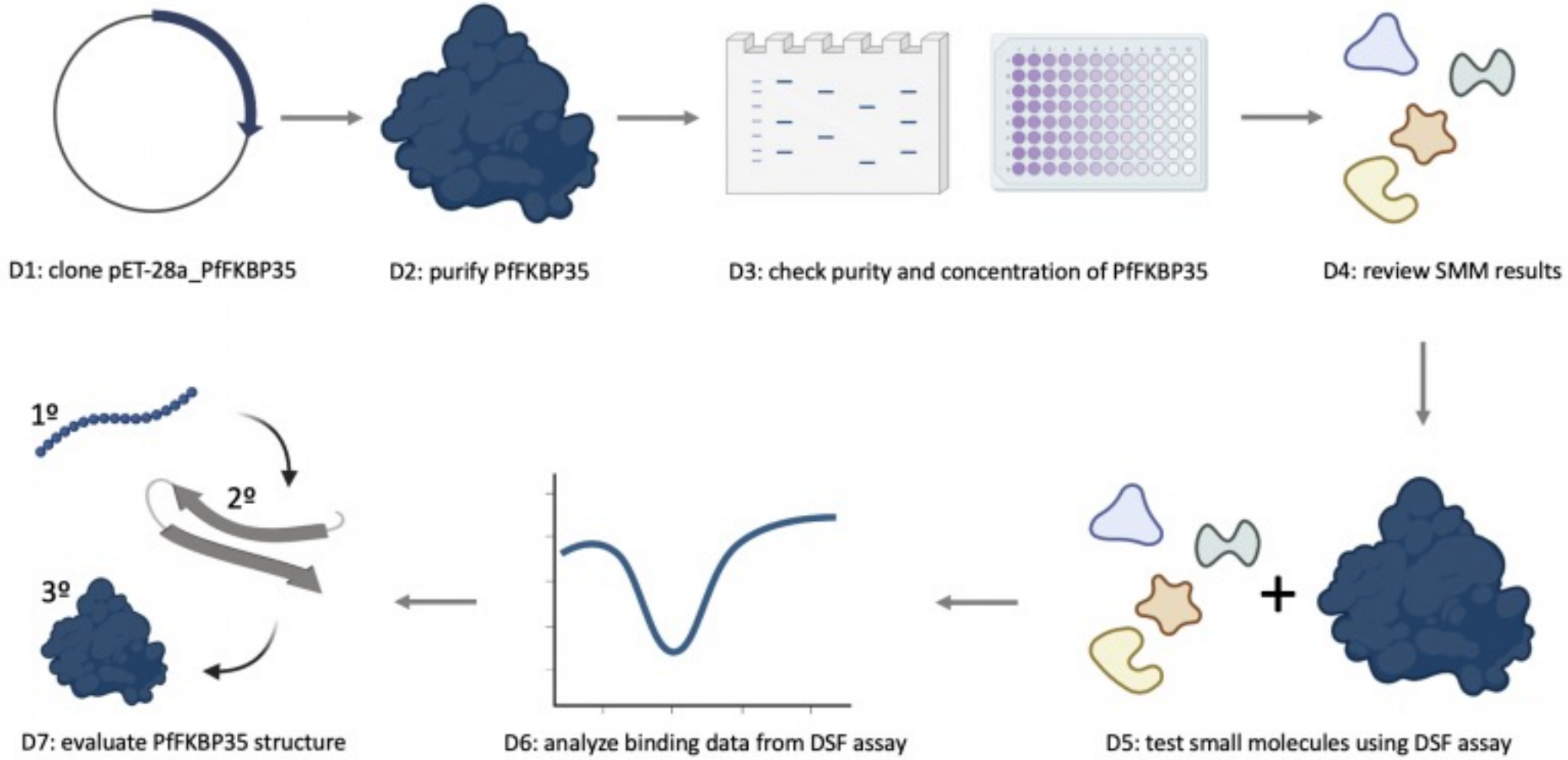
M2D5: Complete the DSF experiment

1. Prelab
2. Set up plates and run DSF experiment
3. Work on Journal Article presentation



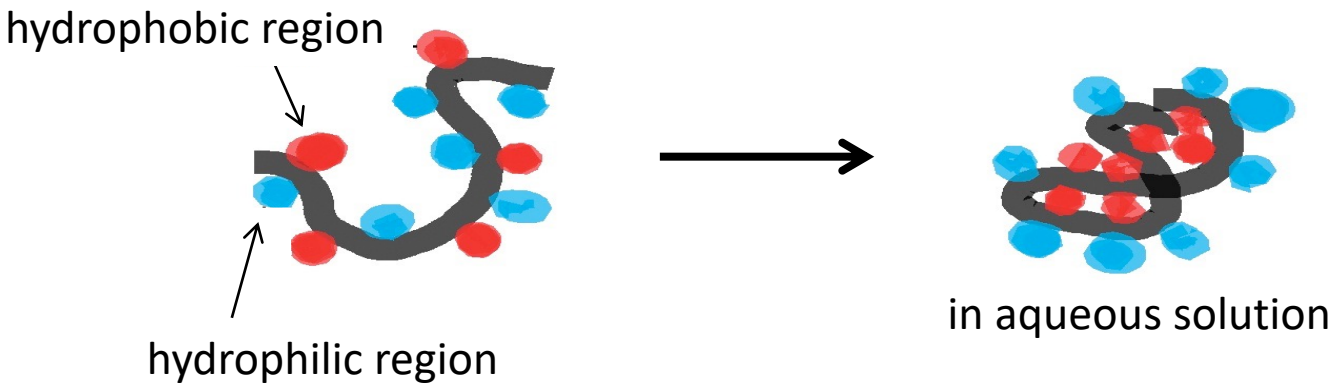
Overview of M2: drug discovery

Research goal: Test small molecules for binding to the *Plasmodium falciparum* FKBP35 protein using a functional assay.



Differential scanning fluorimetry (DSF)

- Probe protein folding by adding a SYPRO Orange 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



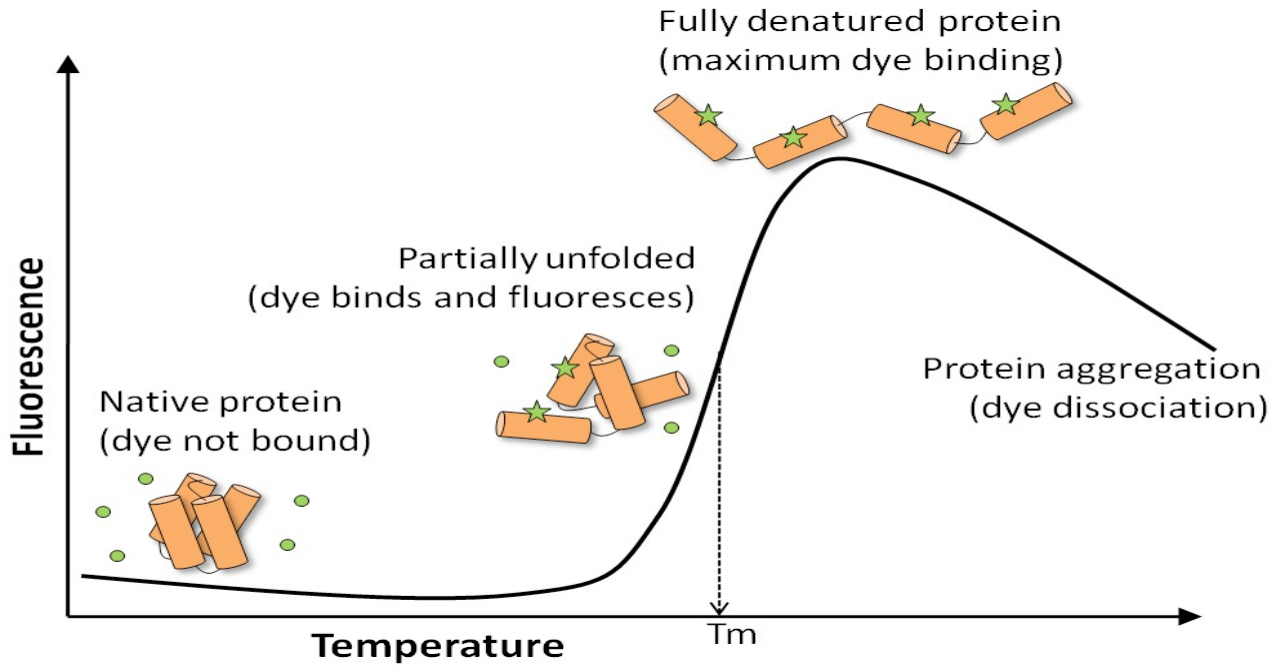
DSF detects proteins destabilizing at high temperatures

- As protein unfolds with temperature increases, SYPRO Orange increasingly binds to hydrophobic regions

- Can calculate a melting temperature (T_M) where 50% of the protein is denatured from quantifying the increase in fluorescent signal

unbound (inactive) dye

bound (active) dye



Differential scanning fluorimetry (DSF) can be used to detect ligand binding

- Ligands bound to protein can stabilize it
  - Slows protein denaturization
  - Seen as a shift in melting temperature
  - Thermal shift assay

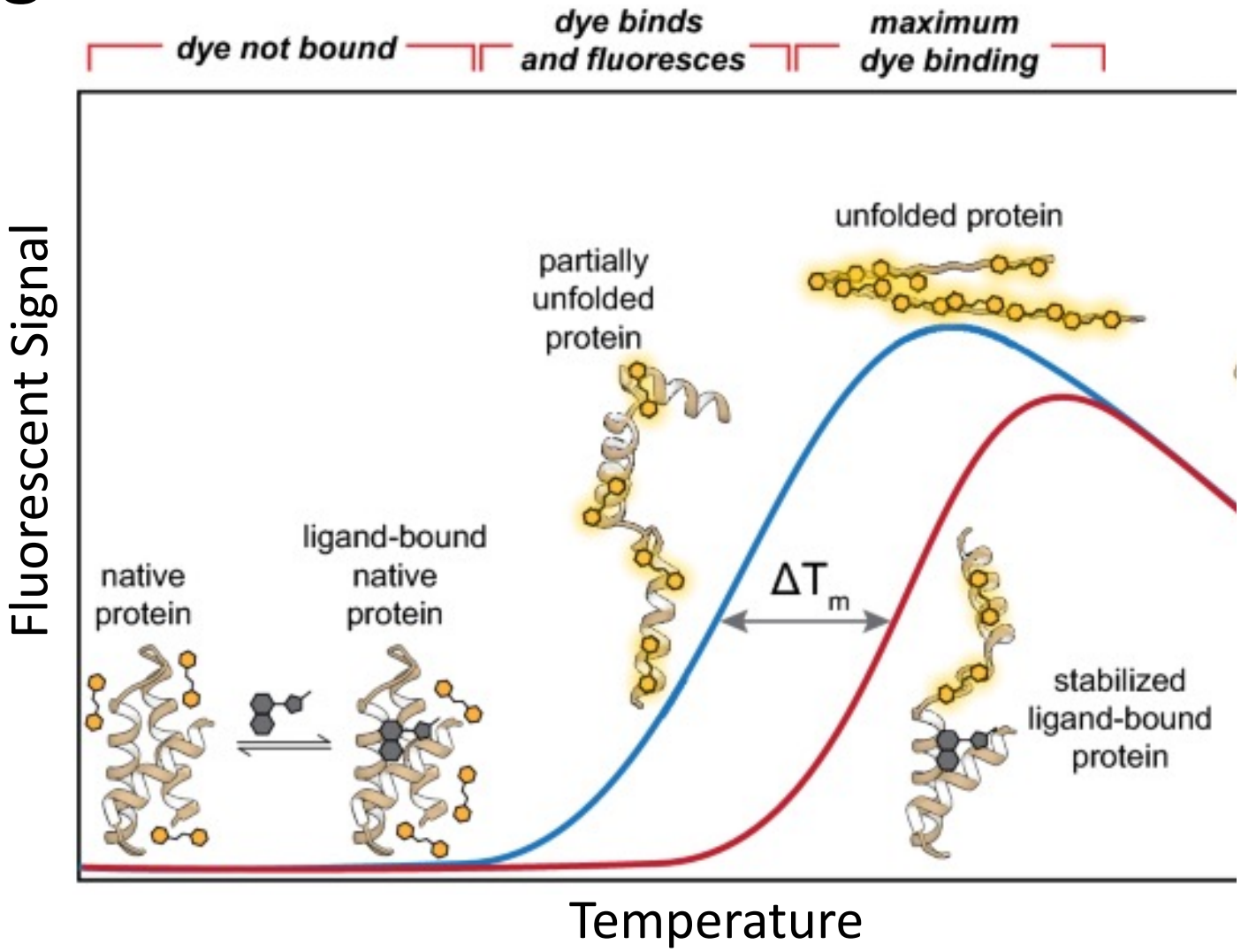
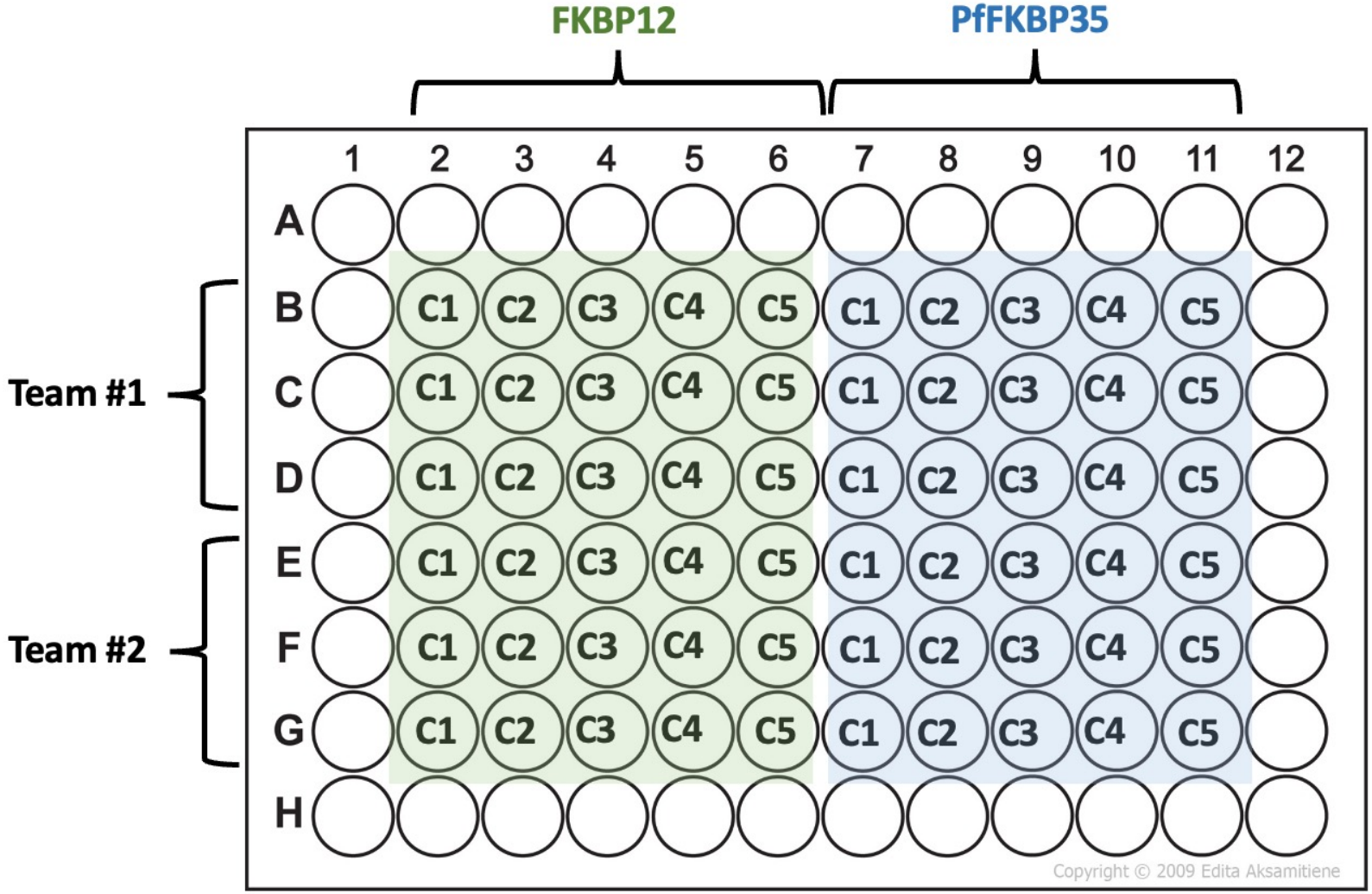


Plate set up for DSF assay

- 2 teams per plate
- 5 compounds per team
  - DMSO
  - 2 SMs to test
  - Rapamycin
  - FK506
- FKBP12 vs PfFKBP35



Compounds for each team

	Compound #1	Compound #2	Compound #3	Compound #4	Compound #5
TR Yellow	DMSO	rapamycin	FK101	FK141	FK150
TR Green	DMSO	FK506	FK150	FK201	FK459
TR Blue	DMSO	rapamycin	FK296	FK401	FK502
TR Pink	DMSO	FK506	FK169	FK189	FK301
TR Purple	DMSO	rapamycin	FK101	FK169	FK502
WF Yellow	DMSO	FK506	FK189	FK301	FK459
WF Green	DMSO	rapamycin	FK150	FK201	FK502
WF Blue	DMSO	FK506	FK141	FK296	FK401
WF Pink	DMSO	rapamycin	FK101	FK169	FK459

Plate groups for DSF

- Plate 1: **Yellow** & **Green**
- Plate 2: **Pink** & **Purple**
- Plate 3: **Blue**

Each plate group should wait for me to tell you before setting up your assay

If you are not setting up a plate– work on your Journal Article presentation!

For M2D6 (Nov 8!)

- Create a data figure of the RE digest results of the PfFKBP35 plasmid
- Write text for results section to accompany figure
  - Research Article will be word doc and paragraphs

- Write a methods section for the Research Article
  - M2D1 (only the confirmation digest) – M2D3

Strategies for the Journal Article Presentation: slides and timing

- Remember that the time limit is strict (10 min +/- 30s) and points will be deducted for each 30 seconds you are over or under

- Be sure to use all tools available to you to convey your message
  - Text can be helpful for you to remember key points
  - Text can also be helpful for the audience to take away key points they might miss in your speaking
  - Avoid gratuitous images – make sure everything occupying the limited real estate of your slides is directly relevant to the story you are telling
  - Make sure all images are explained

Strategies for the Journal Article presentation: data figures

- You can modify figures to simplify them for presentation as long as you do not change the overall conclusion of the data

- Some data like large figures and tables are difficult to present
  - Consider recreating the relevant part of the figure if possible (especially for tables)
- Feel free to pull images from other sources (or the supplemental section of the paper) to help tell your story, just cite your sources

Other questions?