

M2D5: Complete the DSF experiment

1. Prelab
2. Set up plates and run DSF experiment



Homework

Methods

Write a methods section for the Research Article

- M2D1 (**ONLY** the confirmation digest)
- M2D2
- M2D3

- The methods section in your Research Article is worth 20% of that grade

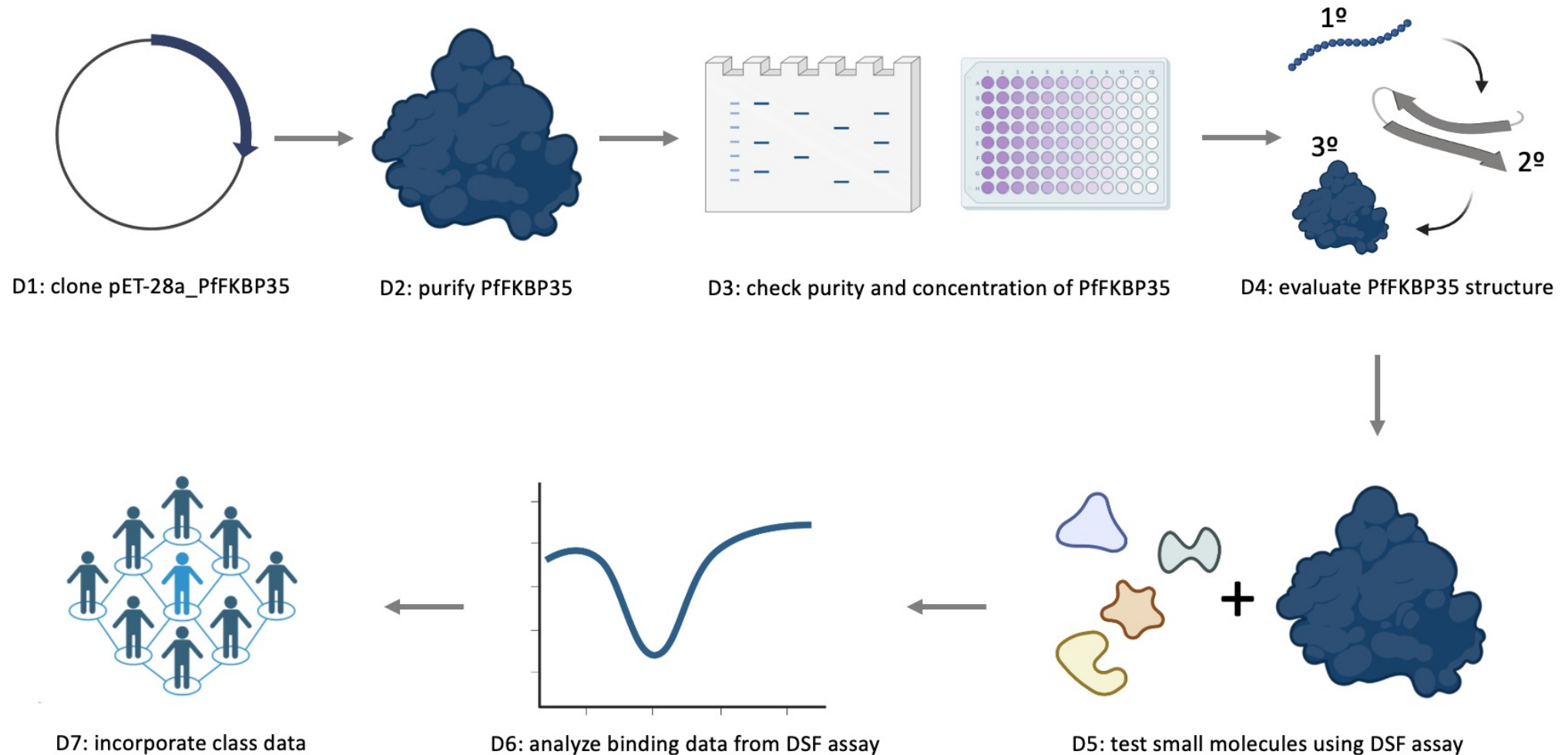
- This is an individual assignment

Lab work

Perform the DSF assay

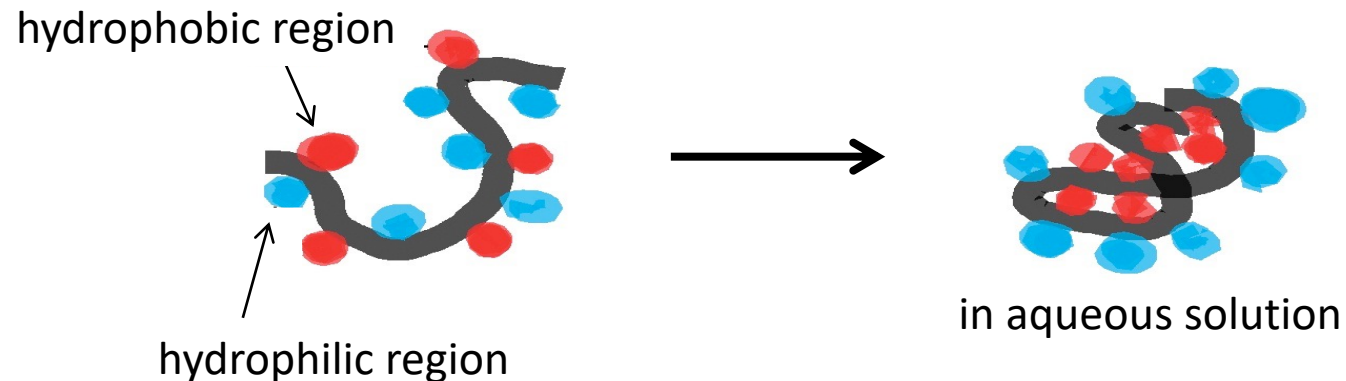
Overview of M2: drug discovery

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



Differential scanning fluorimetry (DSF)

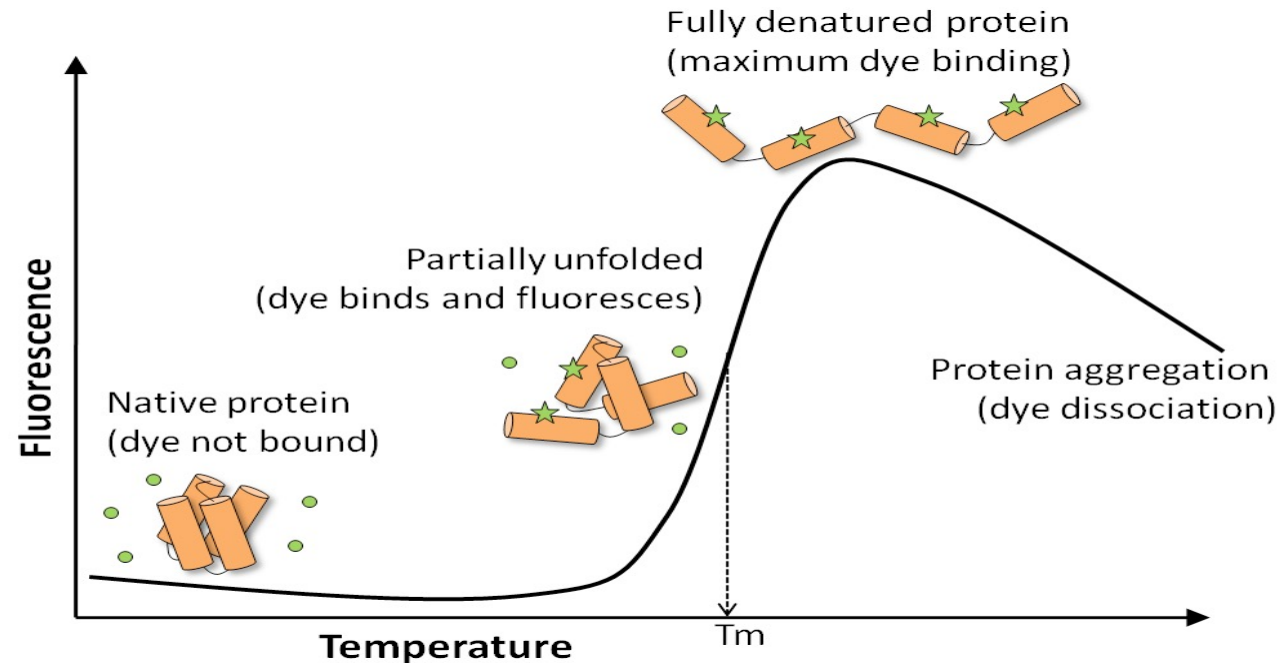
- Probe protein folding by adding a SYPRO Orange dye that interacts with hydrophobic regions of proteins
- If protein is folded, dye is unable to access hydrophobic residues and is inactive (fluorescence quenched in aqueous solution)
- As protein unfolds, dye binds hydrophobic residues and emits fluorescent 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 denaturation
 - Seen as a shift in melting temperature
 - Thermal shift assay

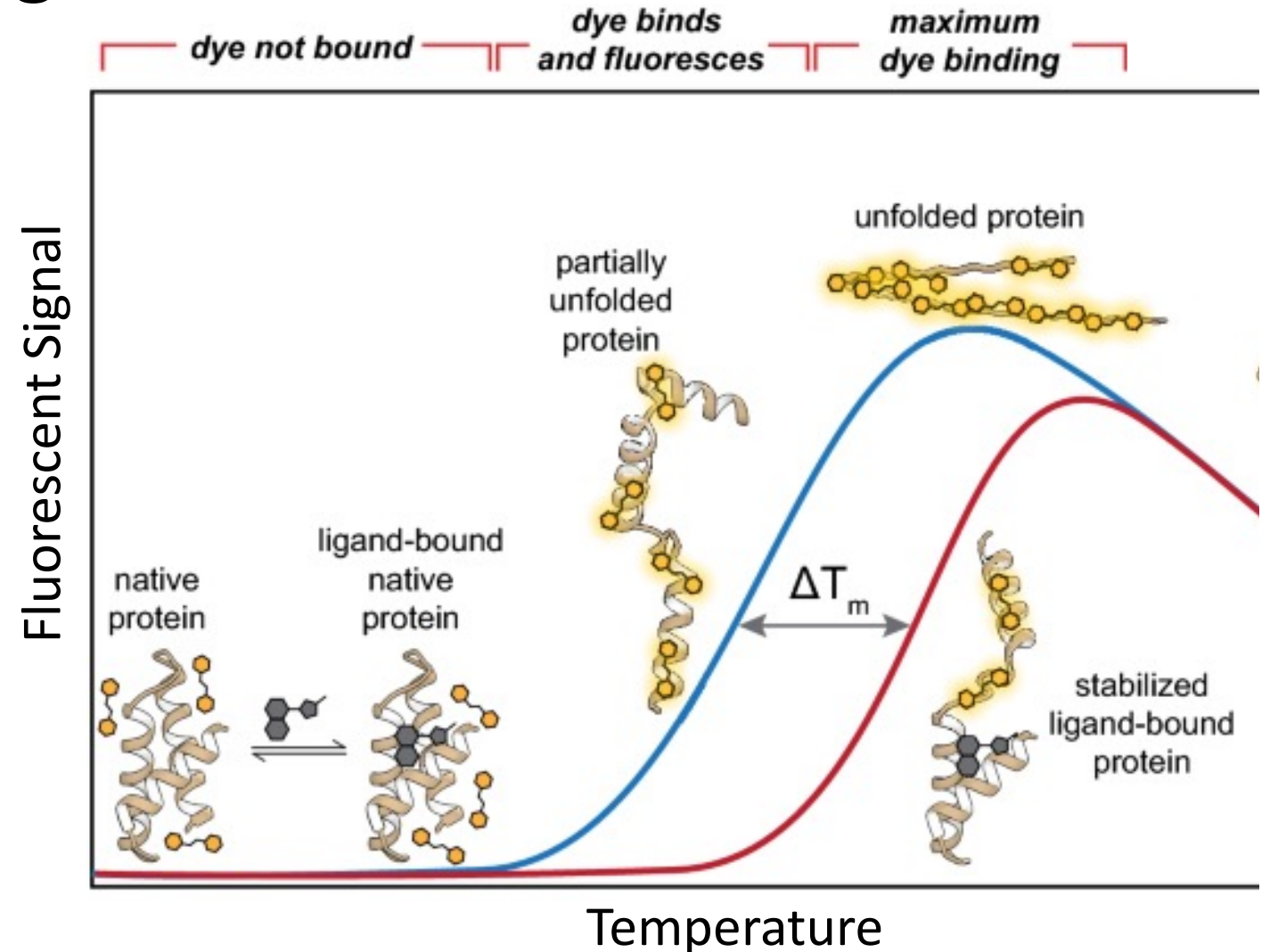
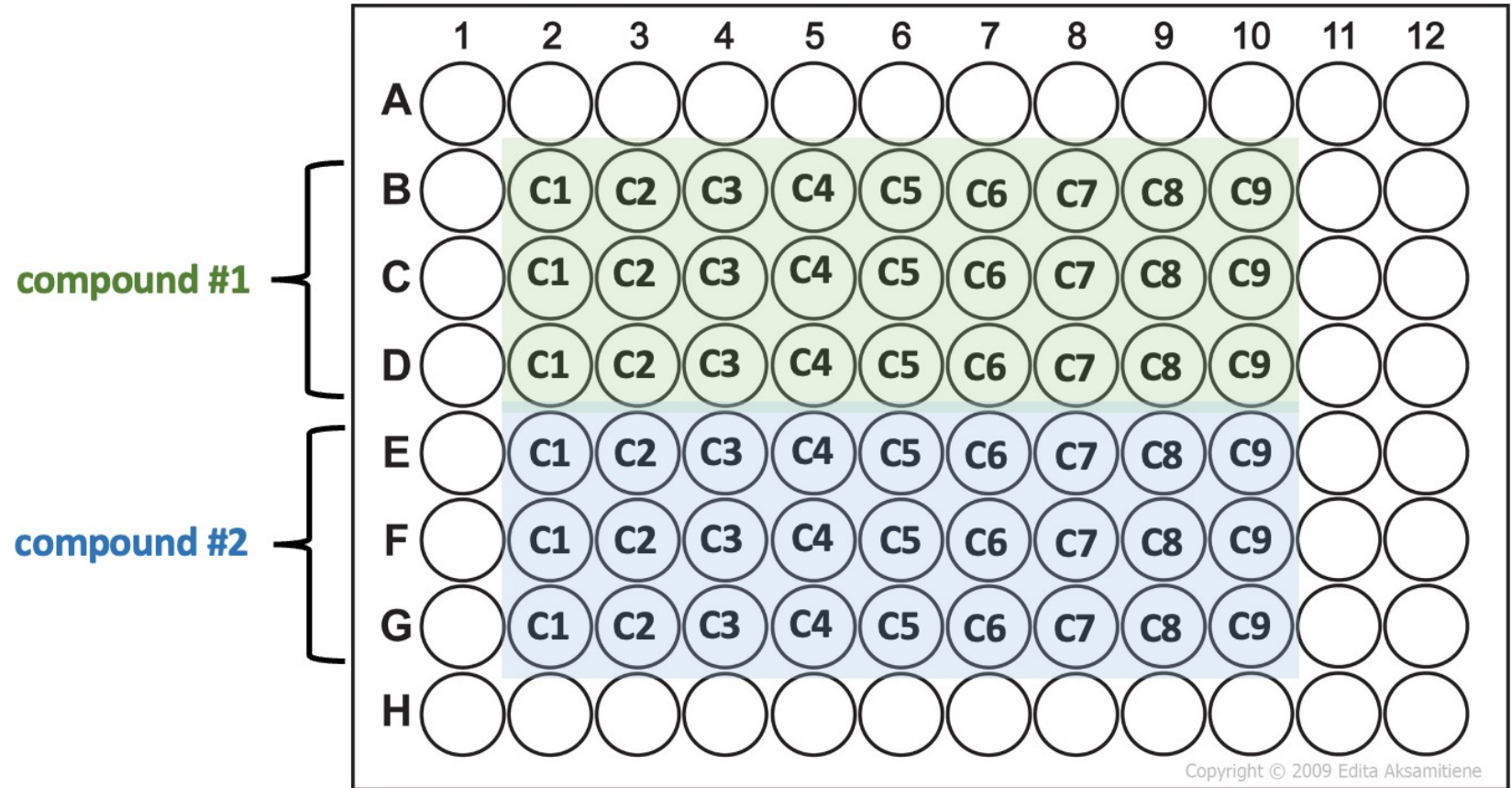


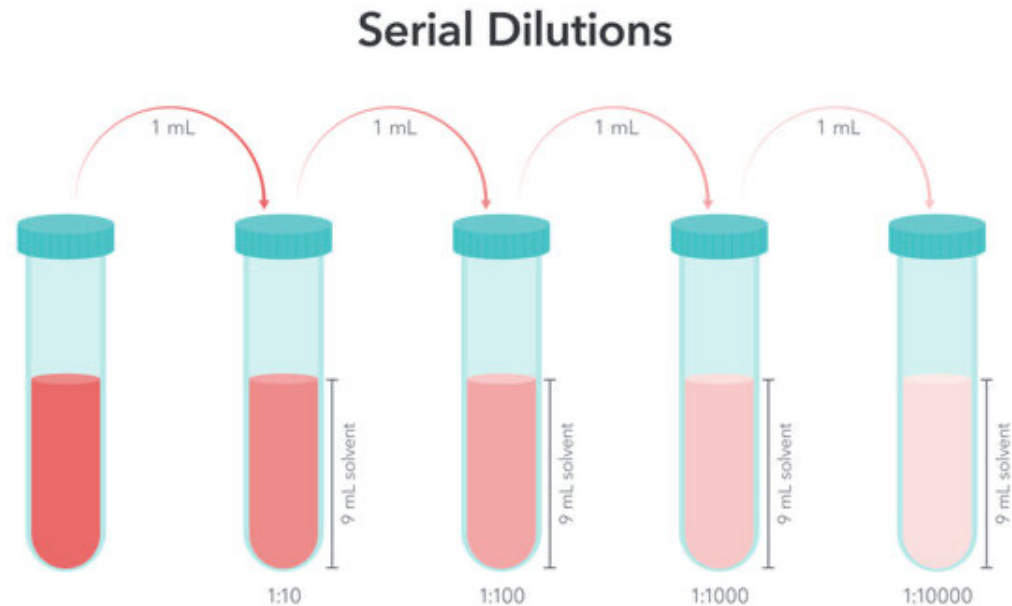
Plate set up for DSF assay

- 2 compounds per plate
- 9 concentrations per compound
 - Each in triplicate



Setting up the serial dilutions of compound

- Different ways to set up serial dilutions



- We have limited compound and excess protein
 - Using protein as the diluent
 - Dilute compound in protein and then continue to dilute compound while keeping protein concentration stable

Notes for DSF conditions

- **Each team member** should set up a serial dilution for **one compound**
 - 2 compounds per plate
- The **control compounds** will be assayed on a **separate plate**
- All **class data** will be **combined**

For today...

- Set up dilutions and assay plate
- Go with Dr. Khan Osman to see DSF plate run
- Spend downtime prepping for Research Article
 - Confirmation digest figure
 - Methods (for homework)