# M2D6: Complete data analysis of functional assay results

- 1. Comm Lab
- 2. Visit equipment used to run DSF
- 3. Prelab
- 4. Analyze DSF data and record  $T_m$  on wiki
- 5. Work on figure outlines for Research Article

THE NEUROBIOLOGY OF WRITING



WWW. PHDCOMICS. COM

### Homework

Create an Overview Schematic and answer questions for the Discussion

### Overview schematics

- Give an overview of the project as a whole
  - Visually represent key concepts/approaches of the project
  - Not much focus on technical details (unlike the experimental schematic)
  - Builds on skills of developing an experimental schematic
- Because it is a figure
  - Include a figure title and caption

## Mod2 Overview (AKA a deliberately terrible overview schematic)

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



### Lab work

Analysis of DSF to identify T<sub>m</sub> shift

#### Mod2 Overview

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



### Analyze files from DSF runs to generate data for Research Article

- 1. Each group has 2 .xml format files.
  - 1. Melt Curve
  - 2.  $T_m$  Calling
- 2. Open them with excel.
- 3. Plot the data from the files according to wiki instructions



#### Plot melt curves and T<sub>m</sub> from DSF data

				Temperatur				
Well 🗖			Ţ		Flu			
x		B2: Sample 14	X	B3: Sample 15				
	20	27.35626734	20	16.39841463				
	20.23	27.24606905	20.23	16.35923957				
	20.31	27.16064967	20.31	16.29594647				
	20.4	27.04181887	20.4	16.24978707				
	20.47	26.98091324	20.47	16.20119984				
	20.55	26.8571273	20.55	16.18912292				
	20.62	26.85207548	20.62	16.18430013				
	20.7	26.78610152	20.7	16.13088011				
	20.78	26.66248474	20.78	16.07510944				
	20.93	26.67263424	20.93	16.04112625				
	21.01	26.62517255	21.01	16.07280797				
	21.08	26.56102698	21.08	16.02662383				
	21.23	26.46291244	21.23	16.00005796				
	21.31	26.45277844	21.31	15.99036847				
	21.39	26.36658467	21.39	15.94670336				
	21.53	26.35138586	21.53	15.89816065				
	21.61	26.31588619	21.61	15.90790488				
	21.76	26.20432895	21.76	15.88368225				
	21.83	26.19259085	21.83	15.85717153				
	21.84	26.15204673	21.84	15.83776293				
	21.98	26.04055041	21.98	15.813561				
	22.13	26.02535184	22.13	15.78445302				
	22.21	25.9696201	22.21	15.7408206				
	22.28	25.91384896	22.28	15.7553921				
	22.41	25,90375014	22.41	15,70689193				

Fluorescence unit

### General plate outlines



#### Melt curve plot example

Temperature       100 uM Compound       No Compound       Image: Compound State	С	D	E	F	G	Н	1	J	К	L	М
29.99       22.8704016       36.3557117         30.05       22.83724515       36.34711833         30.16       22.7914319       36.2756412         30.33       22.7624229       36.25970615         30.39       22.7472188       36.16624032         30.05       22.64675946       36.16647587         30.06       22.53774686       36.01025708         30.072       22.53774686       36.01025708         30.08       22.49573701       35.94972782         31.06       22.39075992       35.8059824         31.16       22.31929394       35.7101708         31.16       22.31929394       35.54022742         31.39       22.1386888       35.54022742         31.39       22.1386888       35.54022742         31.49       22.08378943       35.47014615         31.49       22.08378943       35.54022742         31.39       22.1386888       35.54022742         31.41       21.86445912       35.05498419         31.42       21.86445912       35.05498419         31.52       21.86445912       35.05498419         31.39       21.8221103       34.9502734         32.41       21.86445912       35.05	Temperature	100 uM Compound	No Compound								
30.05       22.83724515       36.34711893       140         30.16       22.7914773       36.30752412       100         30.33       22.764229       36.25970615       100         30.39       22.74172188       36.16647587       100         30.66       22.59646411       36.16647587       100         30.66       22.59646411       36.10125708       36.16647587         30.66       22.59546       36.10625708       36.1025708         31.06       22.3907592       35.80326055       100         31.16       22.3907592       35.80326055       100         31.16       22.3907592       35.805824       100         31.16       22.3907592       35.805824       100         31.18       22.138888       35.5402142       100         31.49       22.08378945       35.36100882       100       100 uM Compound No Compound         31.82       21.86445912       35.515128121       100 uM Compound No Compound       100 uM Compound No Compound         31.92       21.8221103       34.460734       100 uM Compound No Compound       100 uM Compound No Compound         32.19       21.5838267       34.31371508       100 uM Compound No Compound       100 uM Compound No Compound <td>29.99</td> <td>22.87044016</td> <td>36.35577117</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	29.99	22.87044016	36.35577117								
30.16       22.7914373       36.30752412         30.27       22.7914319       36.27264161         30.33       22.7624229       36.25970615         30.39       22.74172188       36.16647587         30.06       22.59646411       36.10613995         30.072       22.53774686       36.01025708         30.06       22.59646411       35.01025708         30.06       22.39075992       35.8059824         31.06       22.39075992       35.8059824         31.16       22.39075992       35.8059824         31.16       22.39075992       35.800882         31.16       22.39075992       35.6402774         31.38       22.1388888       35.54022742         31.39       22.1030567       35.640882         31.49       22.08378945       35.35100882         31.41       21.86445912       35.05088419         31.32       21.82211103       34.96302734         31.38       22.71838367       34.5194747         32.14       21.69699007       34.73513191         32.14       21.69699007       34.4521988         32.21       21.5385867       34.5194747         32.22       21.39848461       34.313715	30.05	22.83724515	36.34711893		140						
30.27       22.7914319       36.27264161       120         30.33       22.7624229       36.25970615       100         30.33       22.7624229       36.166924032       100         30.55       22.64675946       36.16647587       100         30.66       22.59646411       36.10637985       100         30.72       22.53774686       36.01025708       100         30.89       22.49573701       35.94972782       60         31.06       22.3992945       35.8059824       40         31.16       22.31929394       35.71017009       40         31.31       22.13016762       35.47014615       100         31.49       22.08378945       35.3610882       100         31.49       22.08378945       35.35128121       100         31.82       21.86445912       35.0549274       100 uM Compound No Compound         31.82       21.86445912       35.05498419       100       100         31.82       21.86445912       35.05498419       100       100 uM Compound No Compound         31.82       21.86445912       34.8709475       100 uM Compound No Compound       100         32.19       21.66638406       34.66047014       100	30.16	22.7914773	36.30752412					$\sim$			
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31     22.44952261     35.89326055       31.06     22.3907592     35.8055824       31.16     22.31923934     35.71017009       31.27     22.26036547     35.6666697       31.38     22.2138888     35.54022742       31.39     22.13016762     35.47014615       31.49     22.08378945     35.36100882       31.65     22.01211513     35.26052931       31.82     21.86445912     35.0638419       31.82     21.86445912     35.498419       31.92     21.82211103     34.96302734       31.92     21.82211103     34.96302734       32.14     21.66938007     34.73513191       32.19     21.60638406     34.66047014       32.29     21.53858367     34.54194747       32.21     21.36828367     34.54194747       32.22     21.53858367     34.54194747       32.23     21.2678218     34.9987053       32.24     21.32692474     34.19060143       32.25     21.32692474     34.19060143       32.26     21.32692474     34.19060143       32.28     21.20722269     33.98745326       32.39     21.100685     38.89088282	30.89	22.49573701	35.94972782								
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31.27       22.26036547       35.66666697         31.38       22.21388888       35.54022742         31.39       22.10316762       35.47014615         31.49       22.08378945       35.36100882         31.65       22.01211513       35.26052931         31.82       21.86445912       35.05498419         31.92       21.8221103       34.96302734         31.98       21.74181703       34.86047014         32.14       21.66638406       34.66047014         32.19       21.66638406       34.66047014         32.29       21.5388367       34.31371508         32.62       21.32692474       34.19060143         32.72       21.2678218       34.09807093         32.88       21.2072269       33.89088282	31.16	22.31929394	35.71017009		40			/			
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31.92       21.82211103       34.96302734         31.98       21.74181703       34.87099475       Image: Control of the state of the stat	31.82	21.86445912	35.05498419			_	100 uM	Compound	d <u> </u>	o Compo	und
31.98       21.74181703       34.87099475       Image: Constraint of the second seco	31.92	21.82211103	34.96302734				200 4111	oompour.		e compe	GITG
32.14       21.6699007       34.73513191       Image: Constraint of the second of th	31.98	21.74181703	34.87099475								
32.19       21.60638406       34.66047014       Image: Constraint of the second of t	32.14	21.66990007	34.73513191								
32.29       21.53858367       34.54194747       Image: Constraint of the sector of t	32.19	21.60638406	34.66047014								
32.41       21.47072907       34.44521988       Image: Constraint of the system of t	32.29	21.53858367	34.54194747								
32.51       21.39484915       34.31371508       Image: Comparison of the compari	32.41	. 21.47072907	34.44521988								
32.62       21.32692474       34.19060143       Image: Comparison of the compari	32.51	21.39484915	34.31371508								
32.72       21.26738218       34.09807093       Image: Comparison of the compari	32.62	21.32692474	34.19060143								
32.88         21.20722269         33.98745326 <th< th=""> <th< td=""><td>32.72</td><td>21.26738218</td><td>34.09807093</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></th<></th<>	32.72	21.26738218	34.09807093								
32.93 21.1100685 33.89088282	32.88	21.20722269	33.98745326								
	32.93	21.1100685	33.89088282								
	33.03	21.06185613	33.7794664								

## Plot negative first derivative of fluorescence/time to call $T_{\rm m}$ for each compound



- Mark down the temperature at the inverse peak.
  - These are your T<sub>m</sub> values.
- $\Delta T_m = T_m \text{ of } [uM]$ Compound –  $T_m \text{ of } No$ Compound
- Record the T<sub>m</sub> values for your group on the Class Data page for the Wiki

**RFU=** relative fluorescence units

### For the rest of today

- Complete data analysis
- Work on figure outline
- Work on Research Article