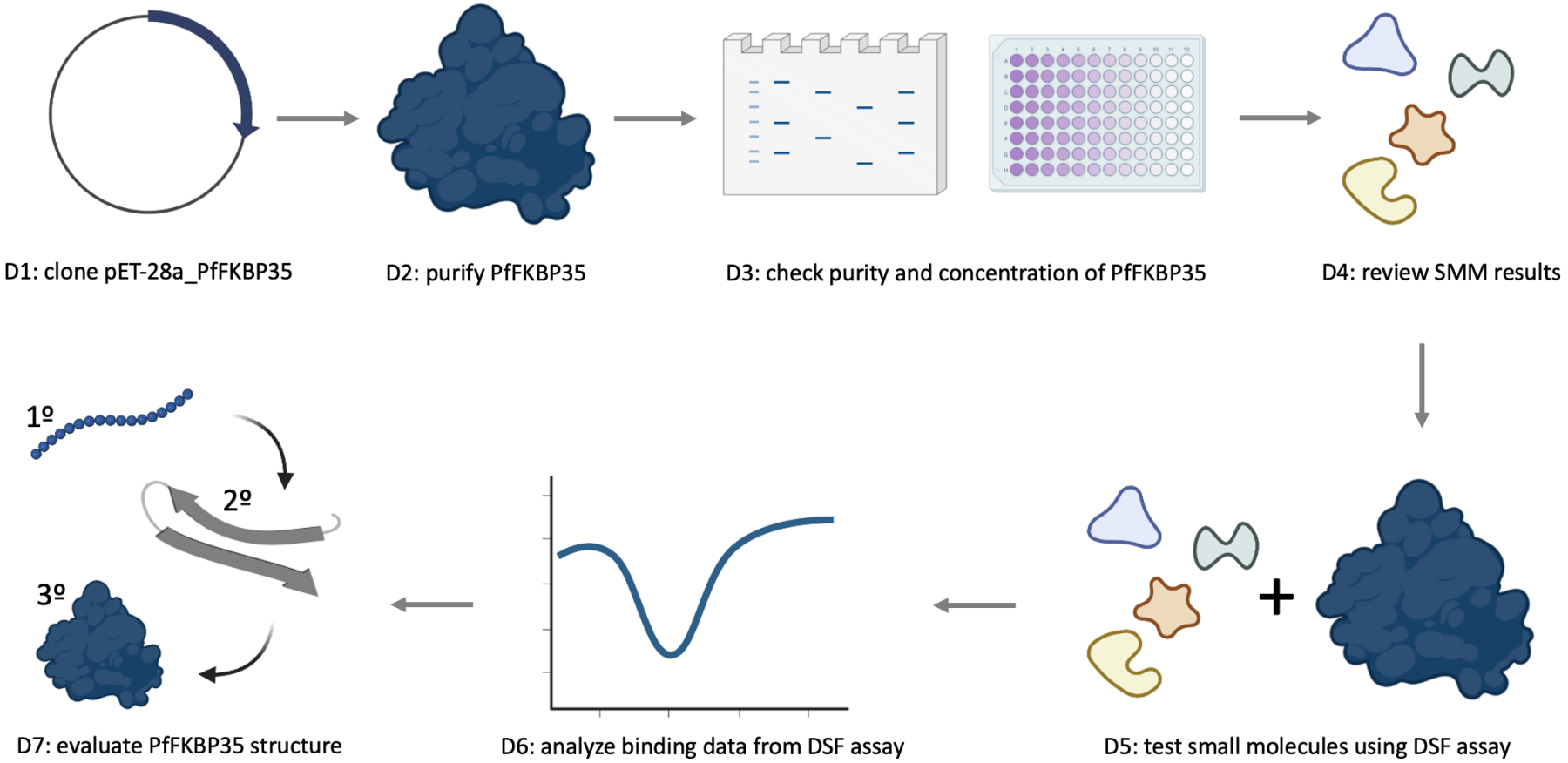
M2D6: Analyze DSF data and outline figures

1. Prelab
2. Analyze DSF data and record T_m on wiki
3. Work on figure outlines for Research Article

Sign up for timeslot to discuss your Journal article Presentation with Noreen
do not sign up during your lab time

Mod2 Overview

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



D1: clone pET-28a_PfFKBP35

D2: purify PfFKBP35

D3: check purity and concentration of PfFKBP35

D4: review SMM results

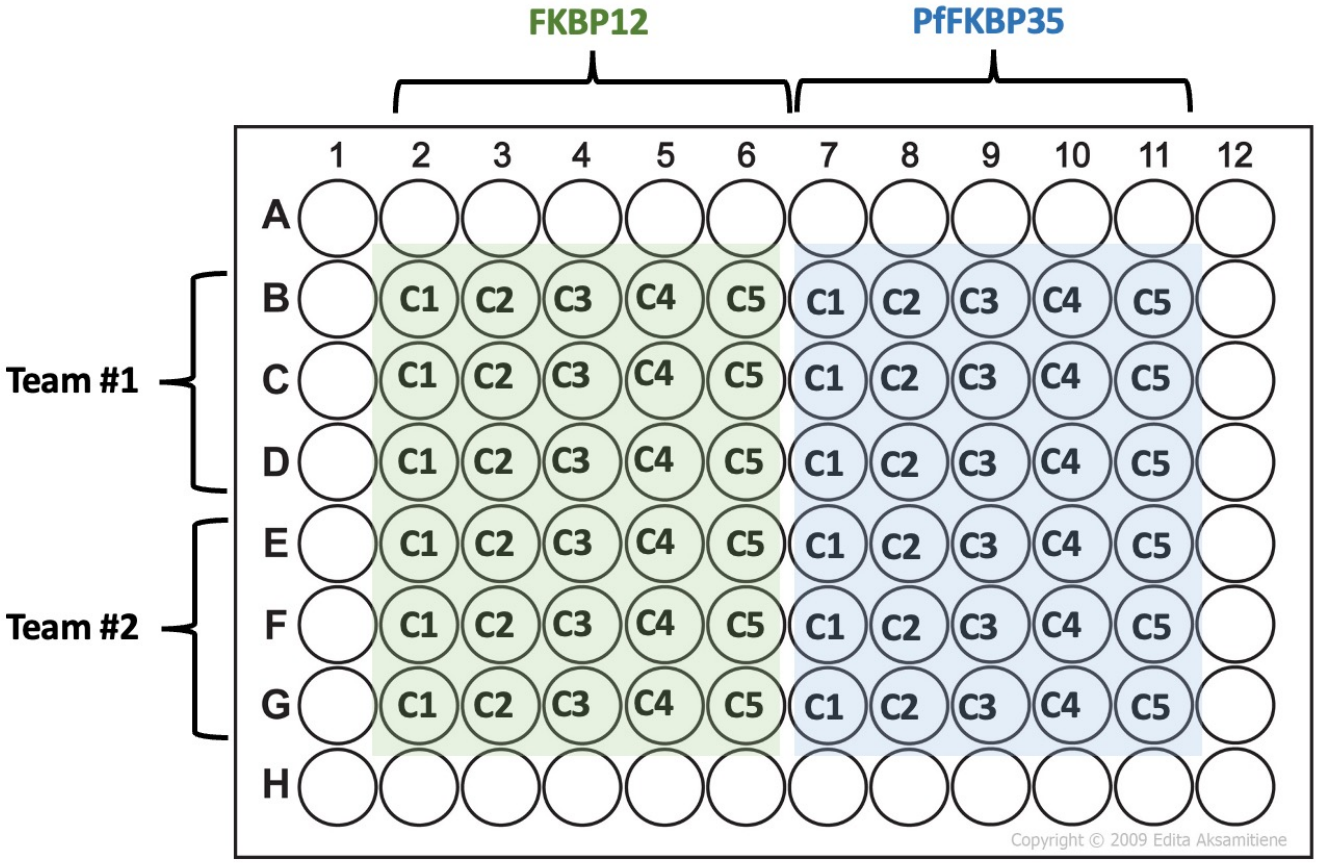
D5: test small molecules using DSF assay

D6: analyze binding data from DSF assay

D7: evaluate PfFKBP35 structure

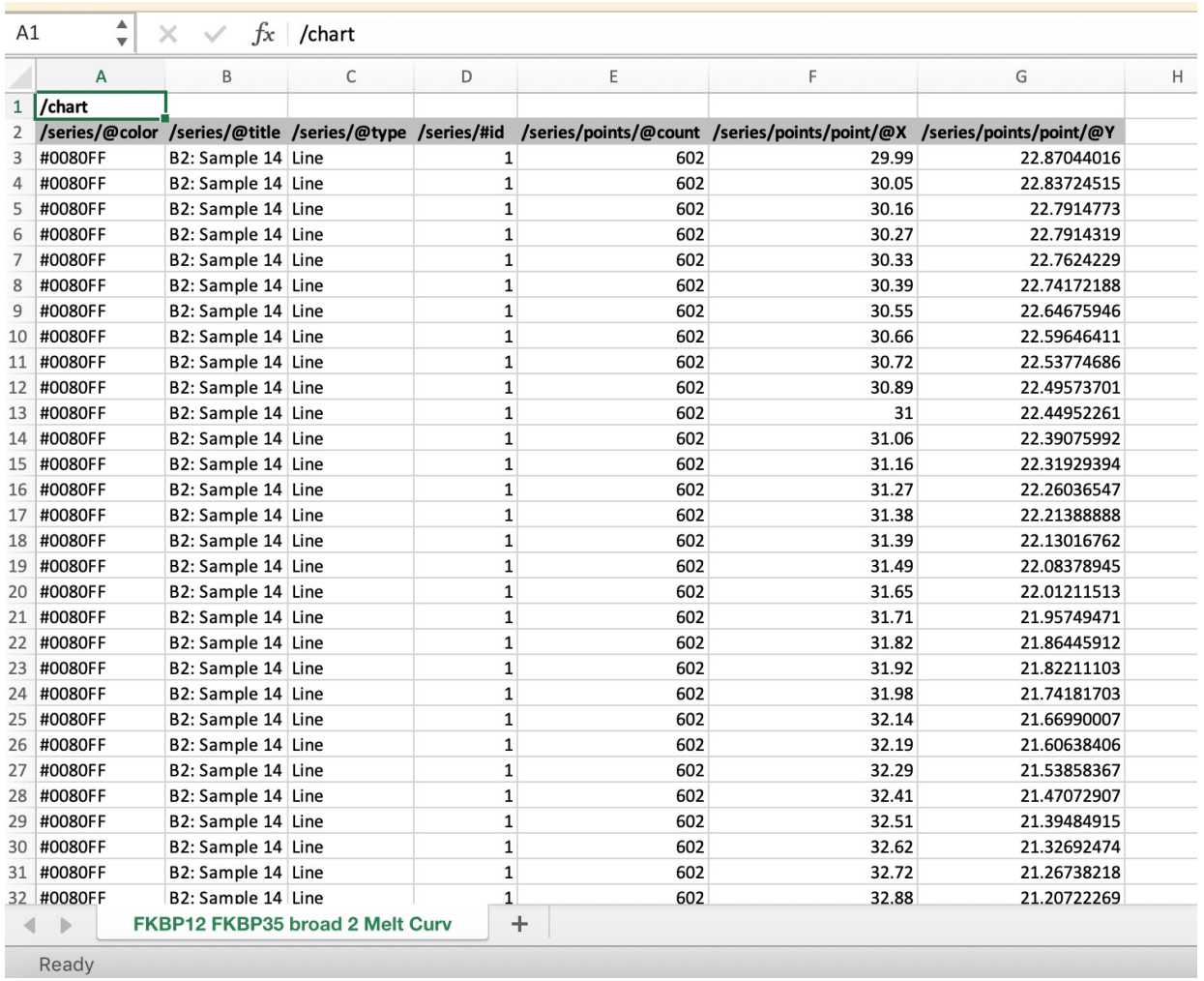
General plate outlines

	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



Analyze files from DSF runs to generate data for Research Article

1. Each group has 2 .xml format files.
   1. Melt Curve
   2. Tm Calling
2. Open them with excel.
3. Plot the data from the files.



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28	#0080FF	B2: Sample 14	Line	1	602	32.41	21.47072907
29	#0080FF	B2: Sample 14	Line	1	602	32.51	21.39484915
30	#0080FF	B2: Sample 14	Line	1	602	32.62	21.32692474
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FKBP12 FKBP35 broad 2 Melt Curv

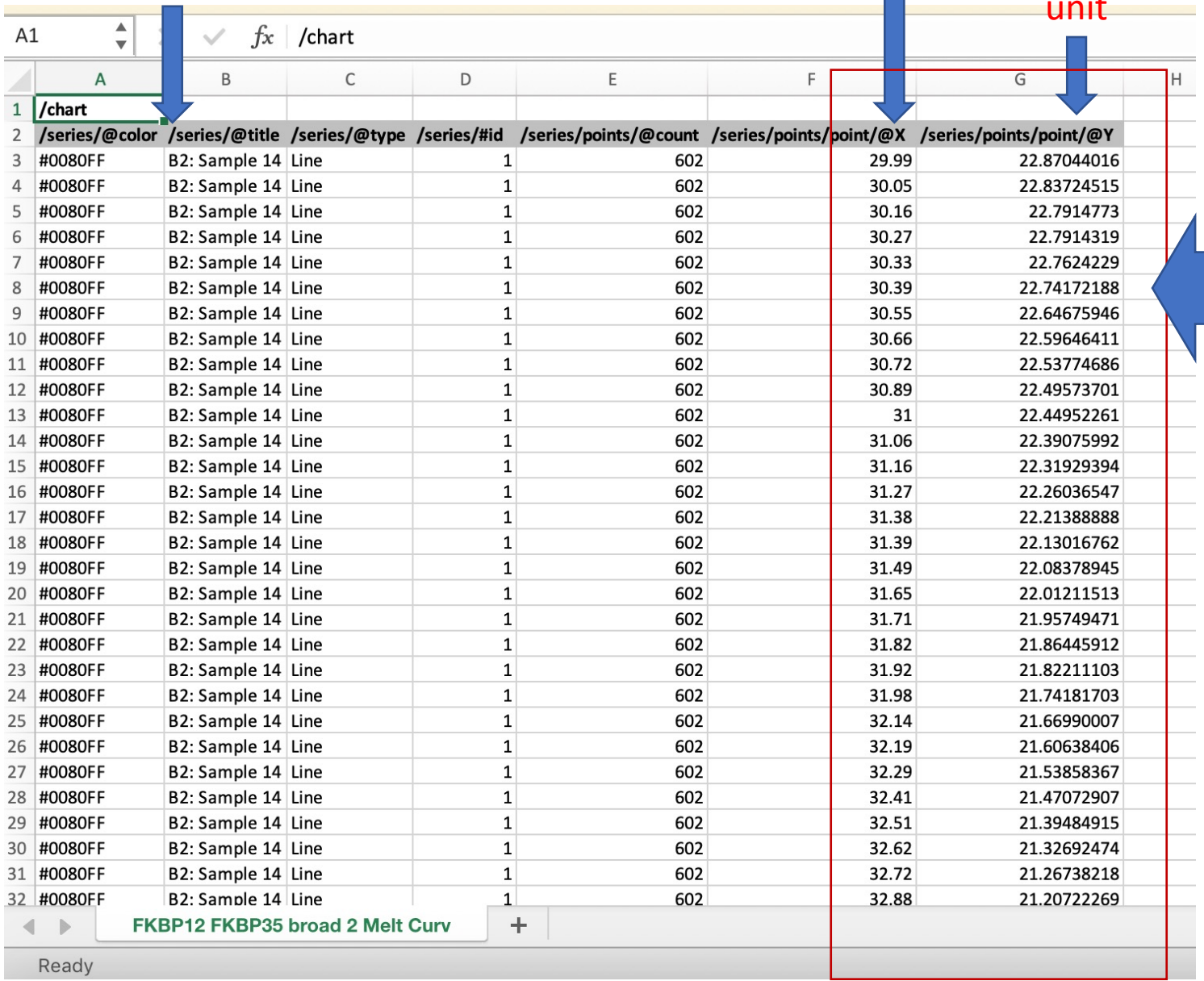
Ready

Plot melt curves from DSF data

Temperature

Well

Fluorescence unit



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30	#0080FF	B2: Sample 14	Line	1	602	32.62	21.32692474
31	#0080FF	B2: Sample 14	Line	1	602	32.72	21.26738218
32	#0080FF	B2: Sample 14	Line	1	602	32.88	21.20722269

FKBP12 FKBP35 broad 2 Melt Curv

Ready

Copy these from individual wells to plot

Average technical replicates together

No statistics

Melt curve plot example

Temperature	100 uM Compound	No Compound
29.99	22.87044016	36.35577117
30.05	22.83724515	36.34711893
30.16	22.7914773	36.30752412
30.27	22.7914319	36.27264161
30.33	22.7624229	36.25970615
30.39	22.74172188	36.16924032
30.55	22.64675946	36.16647587
30.66	22.59646411	36.10613995
30.72	22.53774686	36.01025708
30.89	22.49573701	35.94972782
31	22.44952261	35.89326055
31.06	22.39075992	35.8059824
31.16	22.31929394	35.71017009
31.27	22.26036547	35.66666697
31.38	22.21388888	35.54022742
31.39	22.13016762	35.47014615
31.49	22.08378945	35.36100882
31.65	22.01211513	35.26052931
31.71	21.95749471	35.15128121
31.82	21.86445912	35.05498419
31.92	21.82211103	34.96302734
31.98	21.74181703	34.87099475
32.14	21.66990007	34.73513191
32.19	21.60638406	34.66047014
32.29	21.53858367	34.54194747
32.41	21.47072907	34.44521988
32.51	21.39484915	34.31371508
32.62	21.32692474	34.19060143
32.72	21.26738218	34.09807093
32.88	21.20722269	33.98745326
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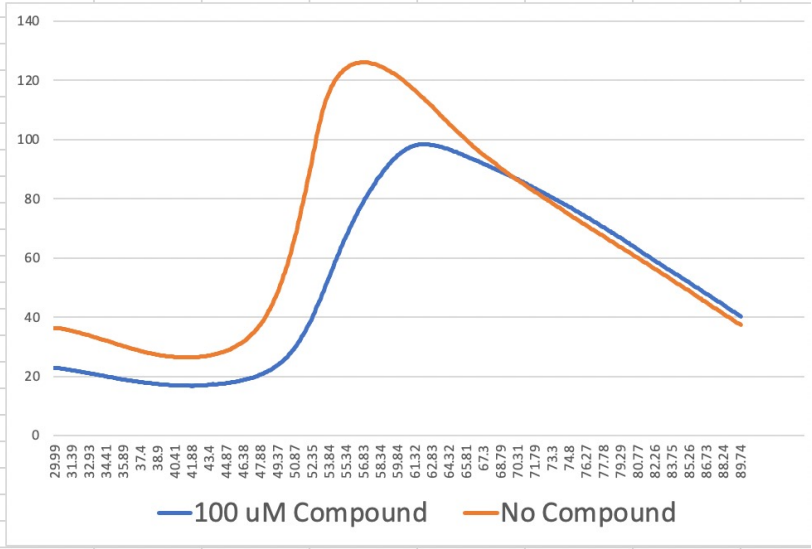
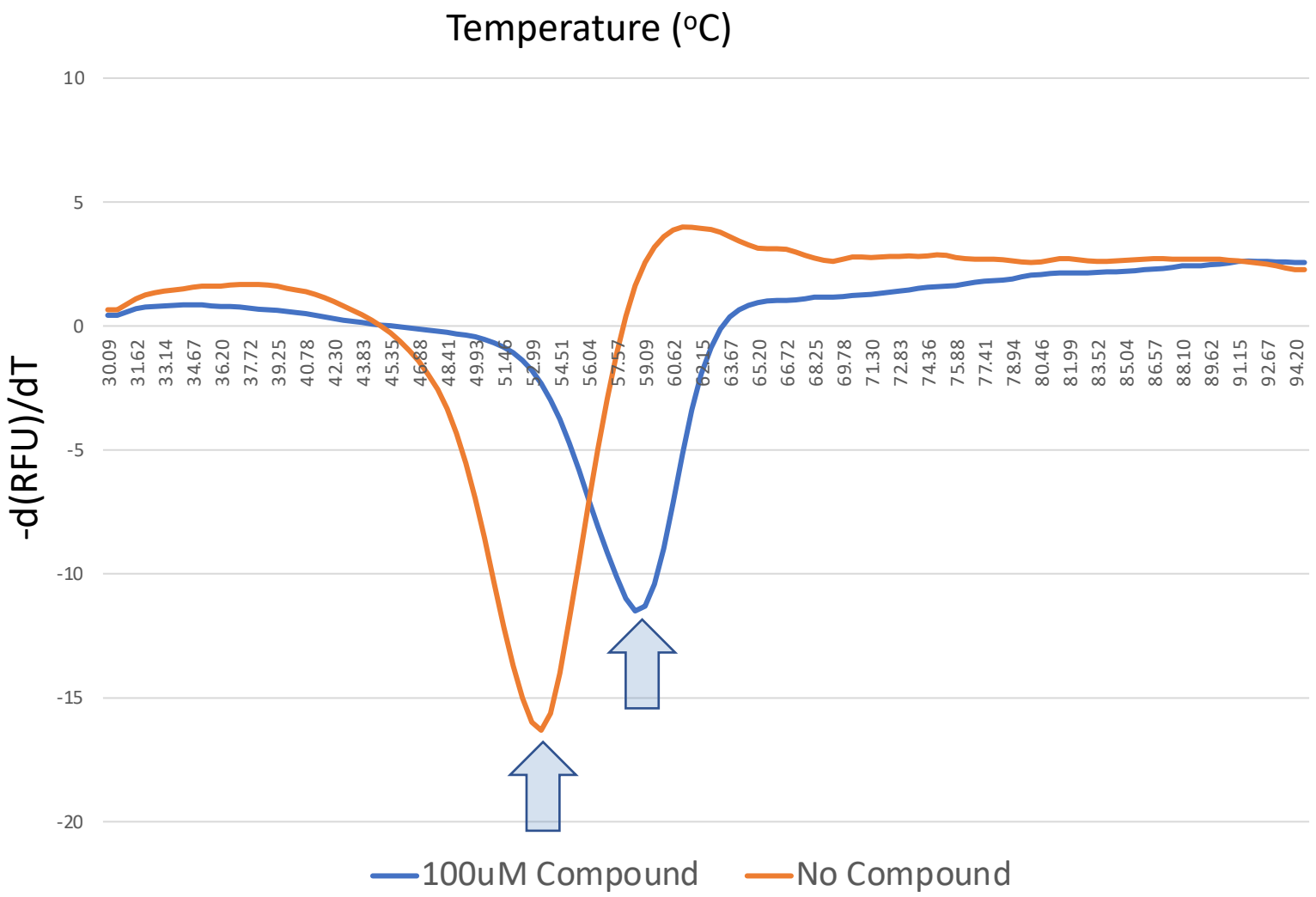


Plot negative first derivative of fluorescence/time to call T_m for each compound



- Mark down the temperature at the inverse peak.
  - These are your T_m values.
- ΔT_m = T_m of 100uM Compound – T_m of No Compound
- Record the T_m values for your group on the Class Data page for the Wiki

RFU= relative fluorescence units

For Today

- Analyze data
- Work on figure outline

For M2D7

- Create overview schematic of project for Mod2
  - High level look at the approach to the project overall instead of a single experiment
- Answer questions to prepare for the discussion section of your Research Article