

# M1D7: Complete data analysis

## Today

1. Quiz
2. Pre-lab discussion
3. Practice statistics exercise
4. Analyze PPlase & DSF data

## Announcements/Reminders:

3/8 (Tmrw!): Lab Notebooks due at 10pm, all entries viewed, esp. M1D1

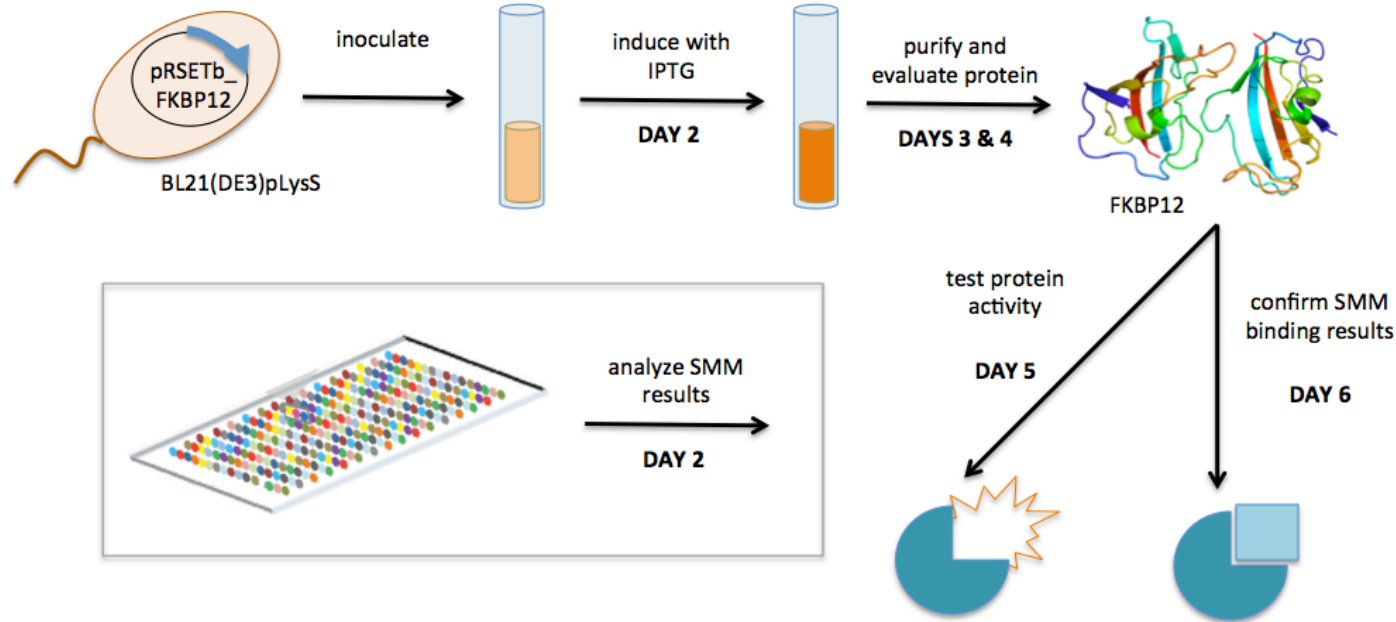
3/10 (Sat): Extra Office Hours, 10am-5pm @ 56-302

3/12: Data Summary due, 10pm

3/17: Mini presentation due, 10pm

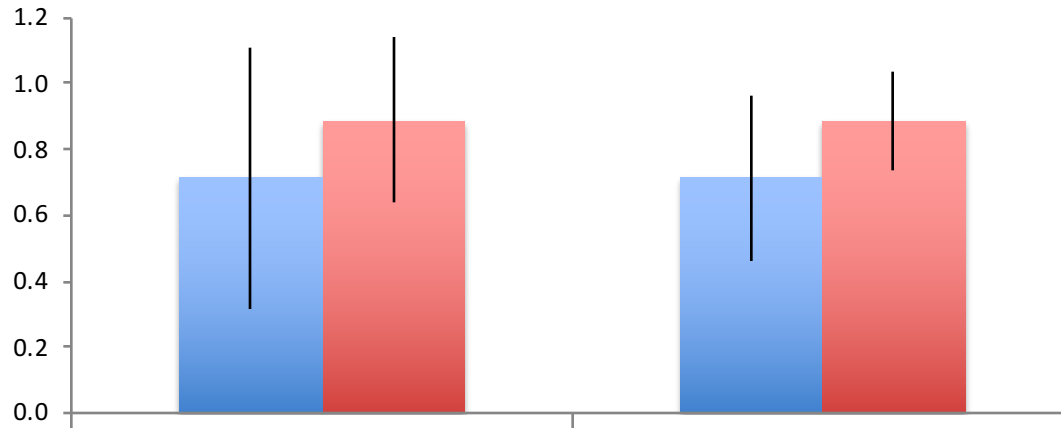
3/18: Blog post due, 10pm

# Overview of Mod1 experiments



# Confidence intervals show the variance in the data set

- At 95% confidence interval, there is a 95% chance that the true mean is within the defined range



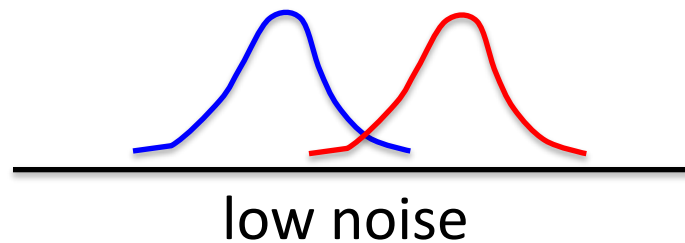
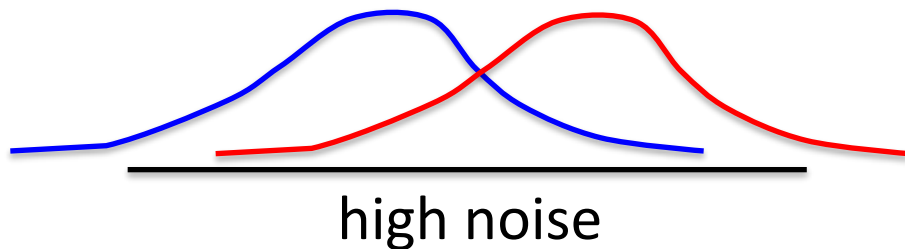
Error bars depict:

95% CI

Standard Deviation

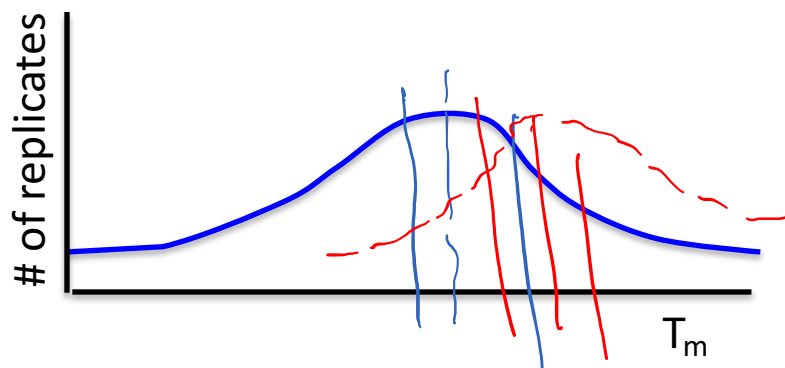
# Student's $t$ -test used to determine if populations are significantly different

- Assume data follows  $t$ -distribution
- At  $p < 0.05$ , there is less than a 5% chance that populations are the same (95% chance that populations are different)
- Examines signal (means):noise (variance) ratio



# Student's $t$ -test used to determine if populations are significantly different

- At  $p < 0.05$ , there is less than a 5% chance that populations are the same (95% chance that populations are different)
- E.g. DSF  $T_{m\_FKBP12} = T_{m\_FKBP12 + Rapamycin}$ ?



If  $p < 0.05$ ,  $T_{m\_FKBP12}$  and  $T_{m\_FKBP12 + Rapamycin}$  are statistically the same/different

no significant difference

significant difference



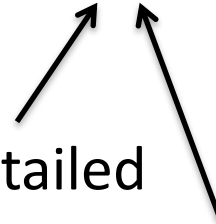
# Calculating Student's $t$ in excel

$$p = TTEST(array1, array2, 2, 3)$$

Use the fewest assumptions:

two-tailed

unequal variance

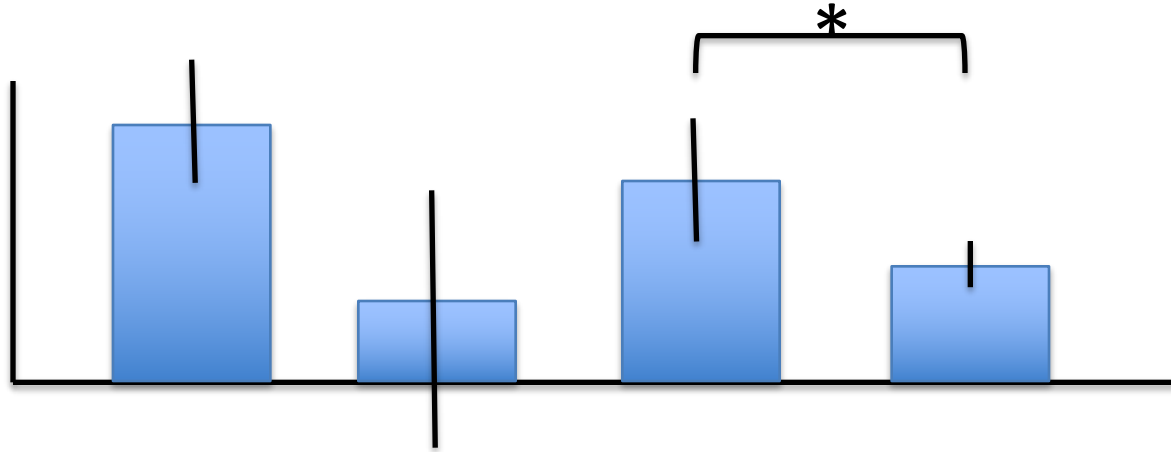


Can only compare two data sets at a time

\*Make sure it is clear on your plots/writing which conditions are being compared

# How will you use statistics in your data analysis?

- Specific activity values calculated from PPlase
- Melting temperatures determined from DSF



What if the data are not statistically significant?

# What were your *expected* results?

- For the PPlase assay:

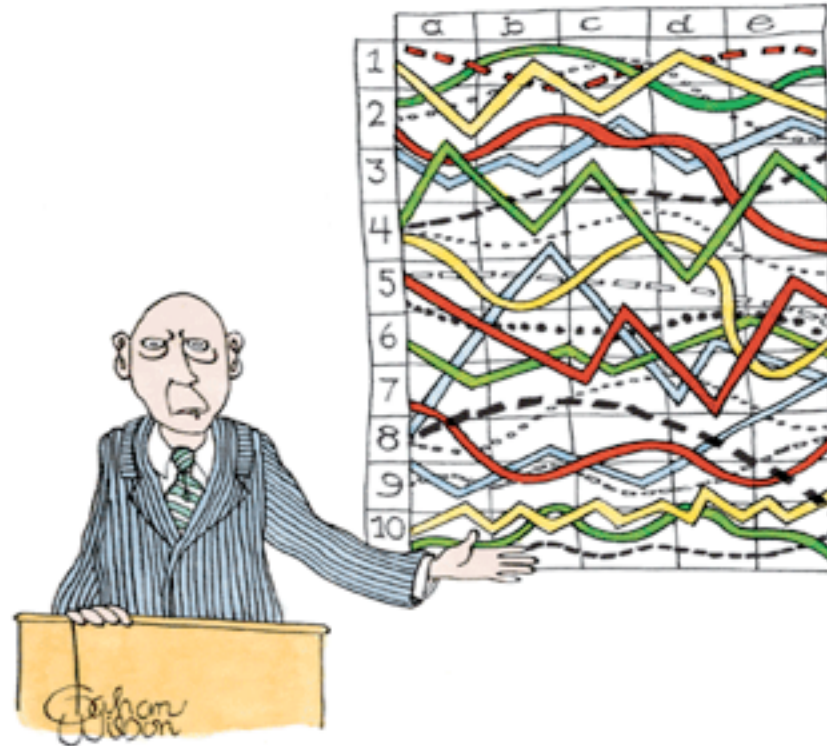
FKBP12 + ligand  $\rightarrow$  decrease activity

- For the DSF assay:

FKBP12 + ligand  $\rightarrow$  increase  $T_m$

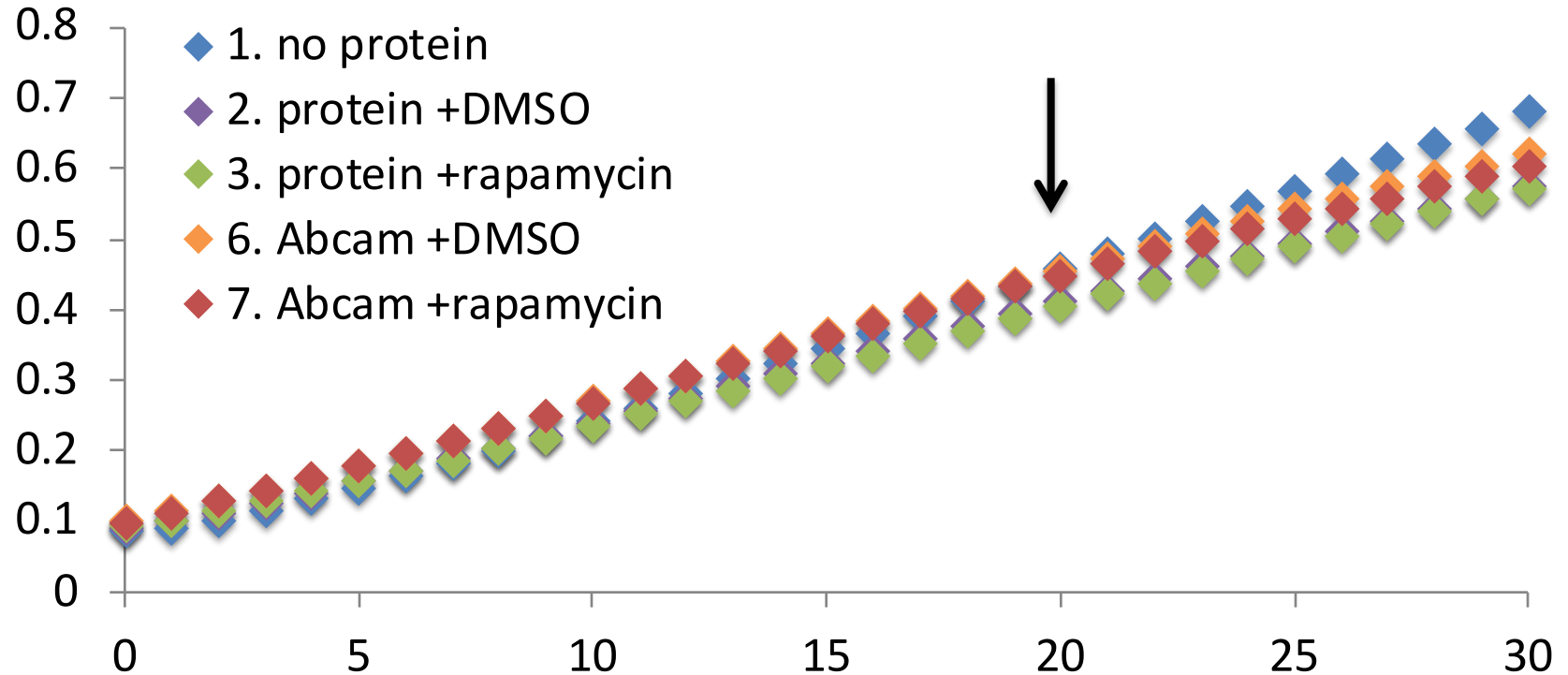


# What were your *actual* results?

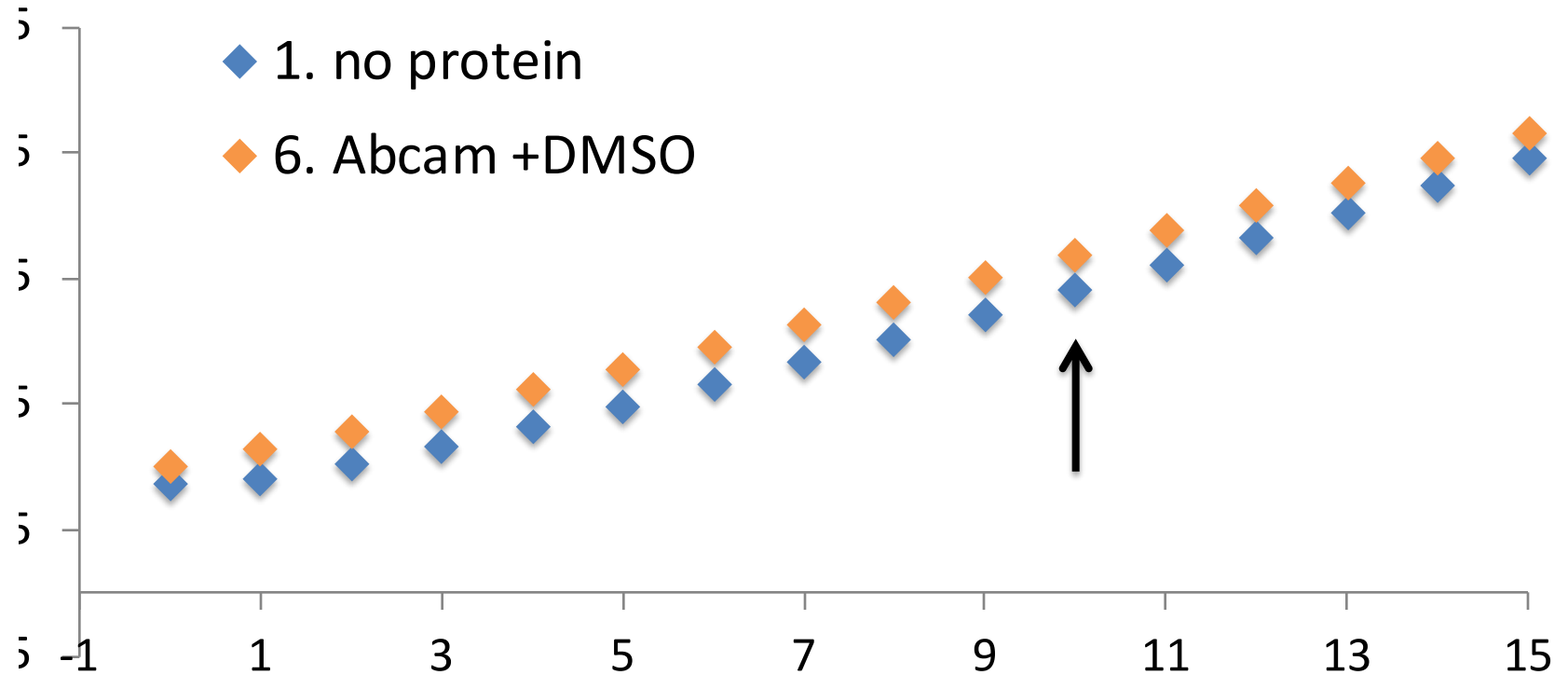


*"I'll pause for a moment so you can let this information sink in."*

# Pooled class data show 'crossover' for test (Abcam) and control samples



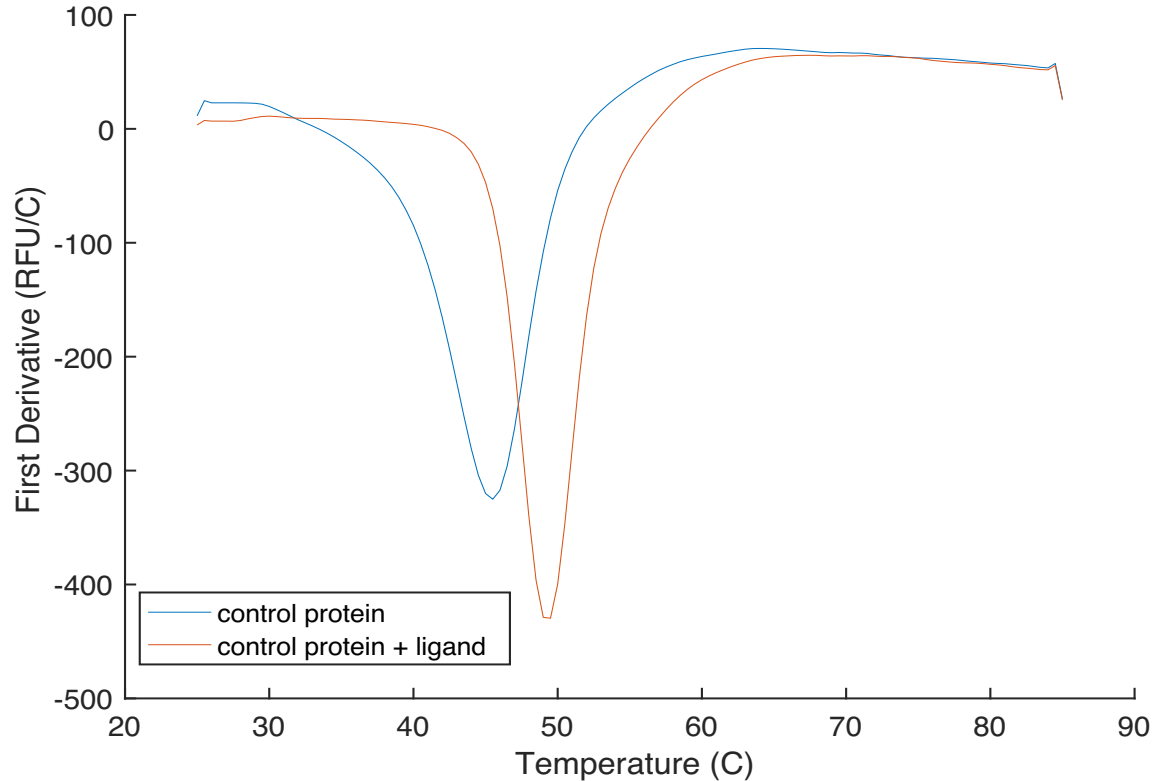
# Let's take a closer look



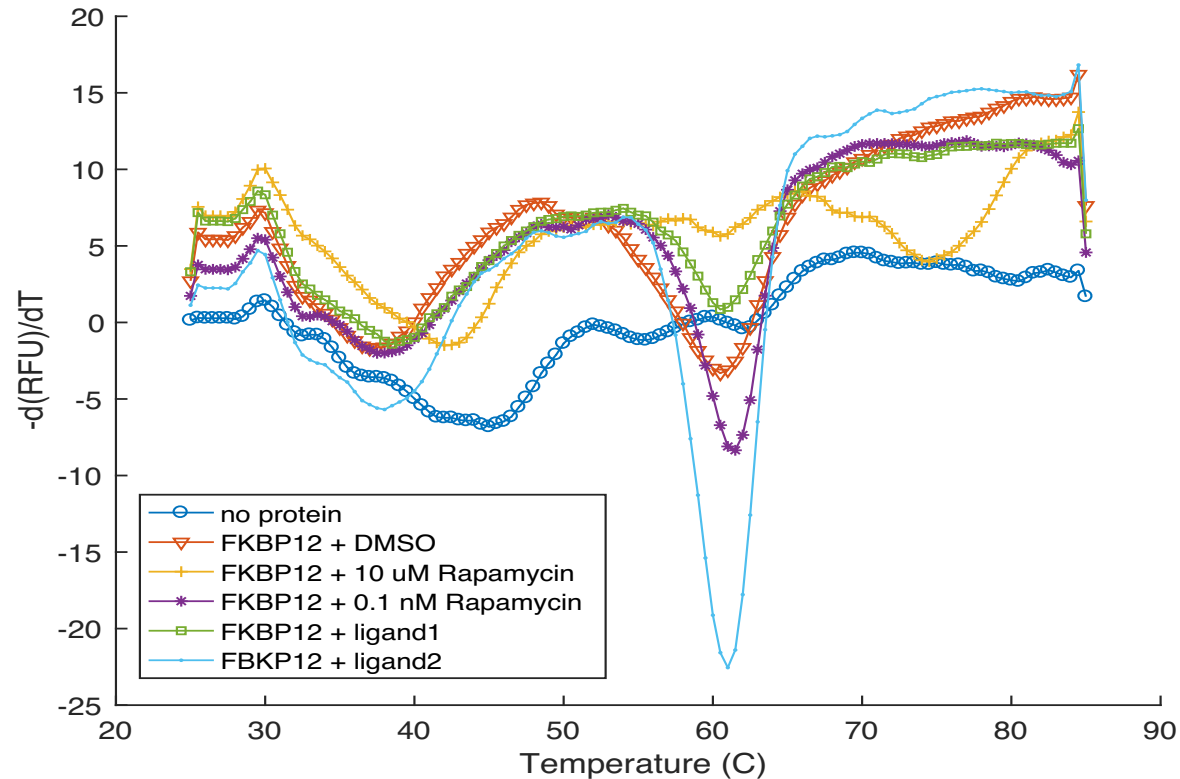
# How will you analyze your PPlase data?

- You will be provided pooled class data
  - Plots for 30 min and 15 min timecourse
- Use  $t = 10$  minutes for final timepoint in specific activity calculations
  - Obtain values from pooled data for Conditions #1, #2, #3, #6, and #7
  - Should still report your individual data!
- Compare your +ligand data to pooled data

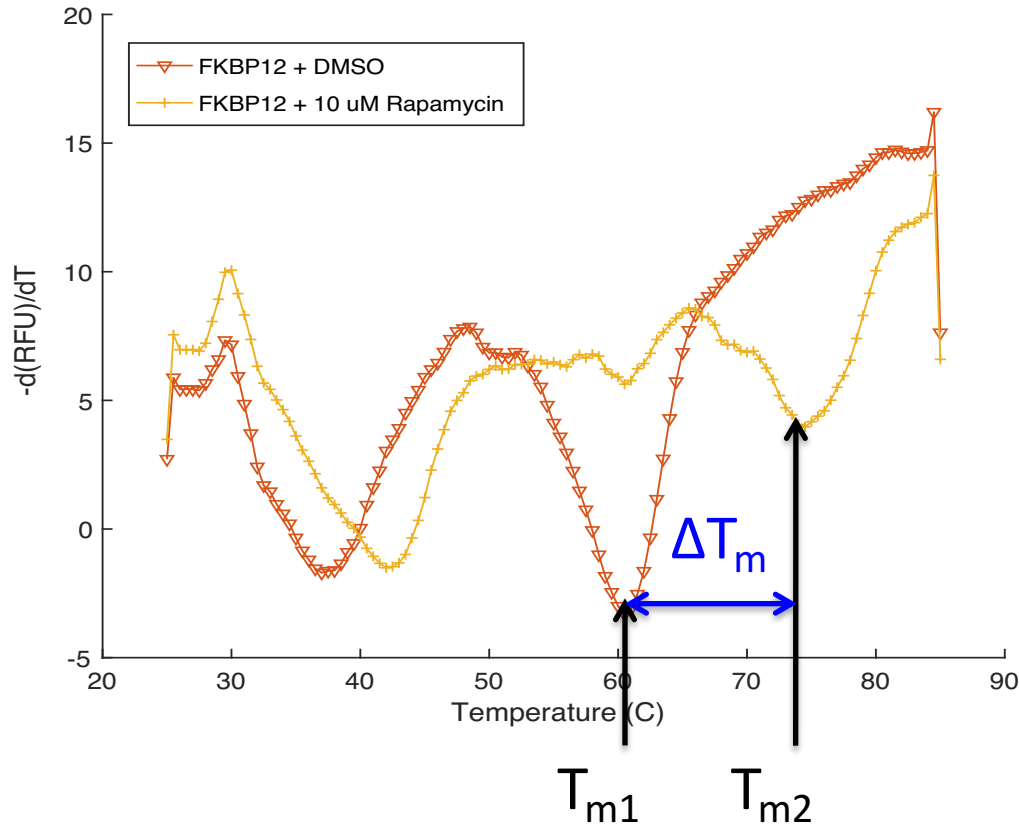
# DSF assay controls look great!



# Now, let's look at your data

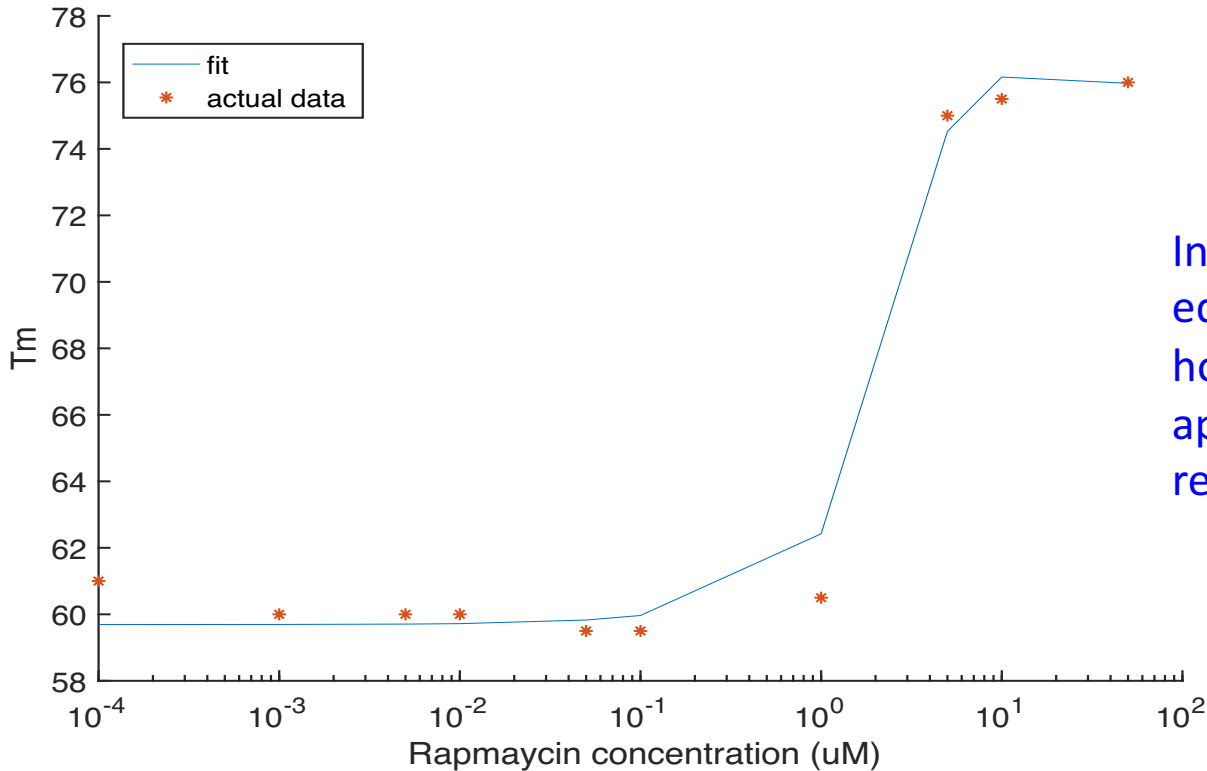


# Define $T_m$ using peak at highest temperature



Find minimum of first derivative in relevant temperature range

# Additionally, calculate the apparent $K_d$ for DSF data analysis



Include model equation (or how you found apparent  $K_D$ ) in report



# How will you analyze your DSF data?

- Complete by-eye validation of  $T_m$  from minimums of first derivatives
- Additional rapamycin concentrations used to calculate apparent  $K_D$ 
  - Pooled class data on wiki
  - Use fit in MATLAB script to back-out value
  - If that fails, use your eyeballs (calculate IC50)

# Be sure to post your data to the wiki!

- For the PPlase assay:
  - Single plot with all curves (t = 0-10 min.)
  - Specific activity calculations for all replicates
- For the DSF assay:
  - Single plot with all first derivative curves—Conditions 3-8 only
  - T<sub>m</sub> values for all replicates
- Should be uploaded by 10 pm tonight!

Be sure all information is clearly labeled in Excel spreadsheet so your classmates can use your data!

# Notes on your Data summary

- Required to use class data
  - PPlase: pooled data AND comparison(s)
  - DSF: pooled  $K_d$  data AND comparison(s)
- Completed with your partner
  - Use individual assignments to generate a ‘polished’ draft
- Follow the format guidelines on the wiki
  - Review the example ‘data’ slide
- Redundancy serves a purpose!

# Today in lab...

- Data analysis

Lab notebook due tomorrow (esp. M1D1)



## For next time

- Read Mod 2 overview and M2D1 introduction
- Prepare for in-class journal article discussion
  - Everyone expected to participate!

# Lastly, some notes on previous homework

- Results slide draft (figure, title, caption, text)
  - Use specific nouns: protein vs FKBP12
  - Describe all data represented in the figure and specifically reference in the text
- Mini-presentation outline
  - Include your hypothesis
  - Be mindful of time limit and focus on key experiments