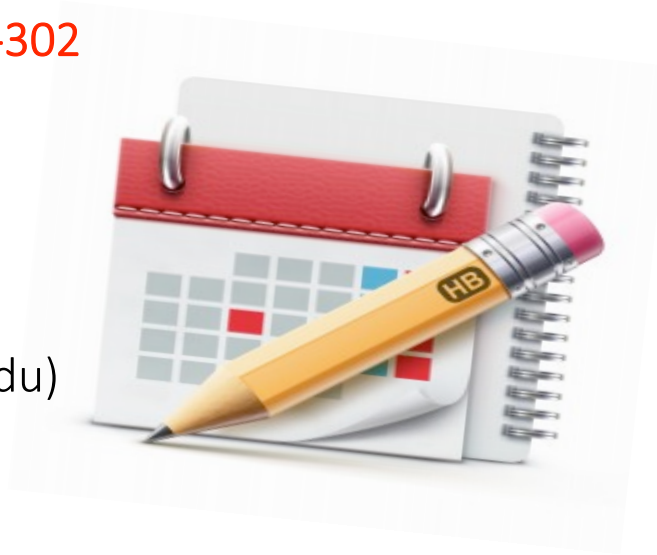
M1D7:
Complete data analysis

QUIZ

1. Prelab discussion
2. Complete statistics practice exercise
3. Analyze PPIase and DSF results

Important due dates are approaching!

- Data summary (15%)
  - completed in teams and submitted via Stellar
  - draft due 3/11 at 10p, final revision due 3/25
  - format in bullet points
  - Extra Office hours Saturday, March 9 12-5p in 56-302
- Mini-presentation (5%)
  - completed individually and submitted via Gmail
  - due 3/16 at 10p
- Notebook (part of 10% Homework and Notebook)
  - M1D4 due 3/7 at 10p via email (mgold01@mit.edu)
- Blog (part of 5% Participation)
  - due 3/17 at 10p via Blogspot



What are your experimental results?

Confirmation digest

Purified FKBP12 → test purity/concentration

Tested activity → PPIase

Tested binding → DSF

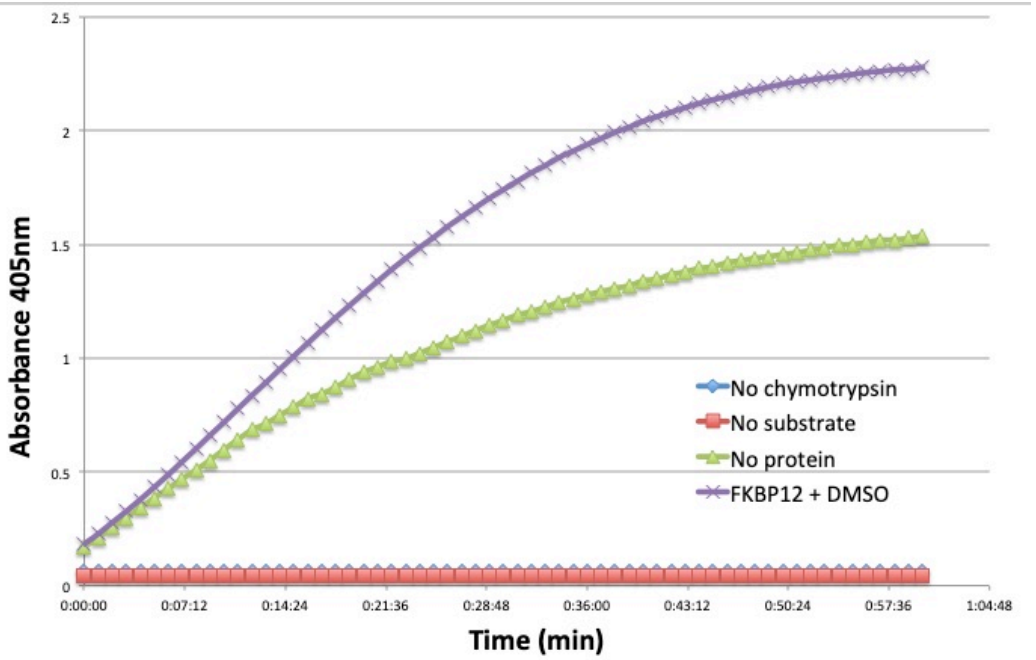
What do your results mean?

- For the confirmation digest:
- For the SDS-PAGE gel and BCA assay:
- For the PPIase assay:
- For the DSF assay:

How will you analyze your PPIase data?

- What is the expected result?

- Plot A_{405} / time
- Calculate specific activity of FKBP12



Quantify the specific activity of FKBP12

$$\text{Specific activity} = \frac{(\Delta A_{405\_Test}/\#\text{min} - \Delta A_{405\_Blank}/\#\text{min})(\text{rxn volume})}{(\text{volume of FKBP12})(\varepsilon_{pNA})}$$

ε_{pNA} (extinction coefficient for pNA) ~ 9.3 mM^{-1}

- Convert FKBP12 volume to mg using known concentration
- Units for specific activity = nmol substrate/min/mg of protein

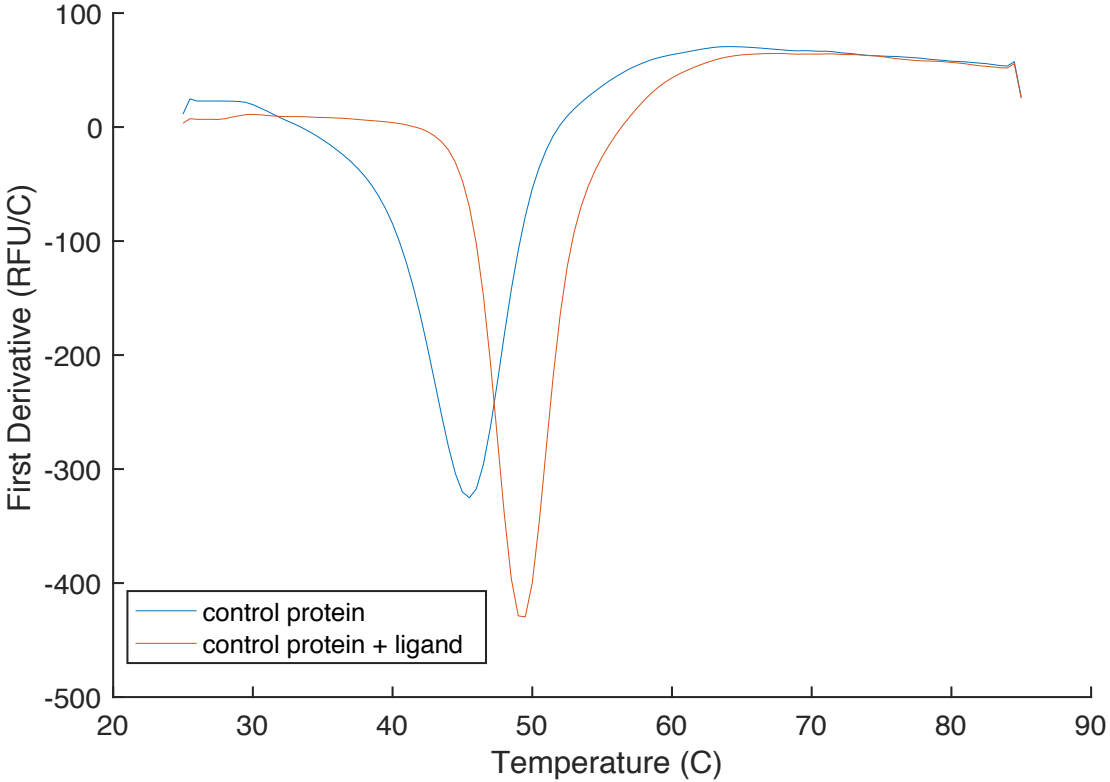
How will you analyze your DSF data?

- What is the expected result?

- Confirm assay / experimental conditions
- Determine T_m values
  - Identify / calculate thermal shifts, if present
- Calculate apparent Kd for FKBP12:rapamycin

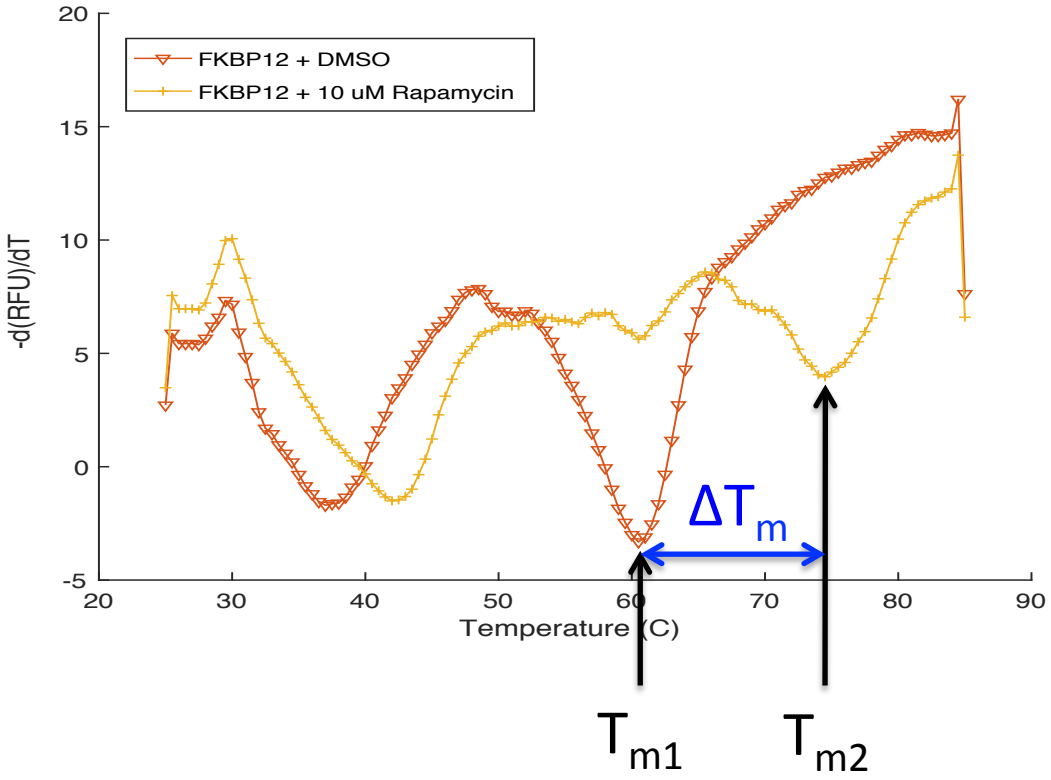
DSF: Confirm assay / experimental conditions

- Control protein:ligand included
  - Protein ONLY: A2, A3, A4
  - Protein + Ligand: B2, B3, B4



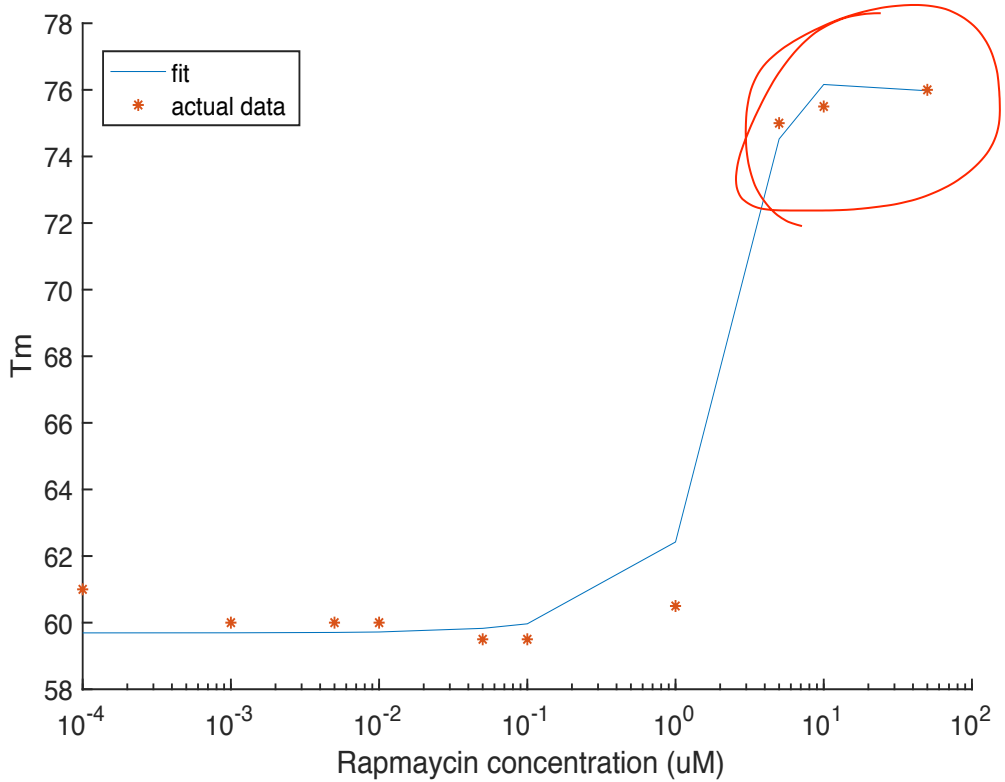
DSF: Determine T_m values

- Find minimum of first derivative in relevant temperature range
- Compare between FKBP12 and FKBP12 + rap / ligands

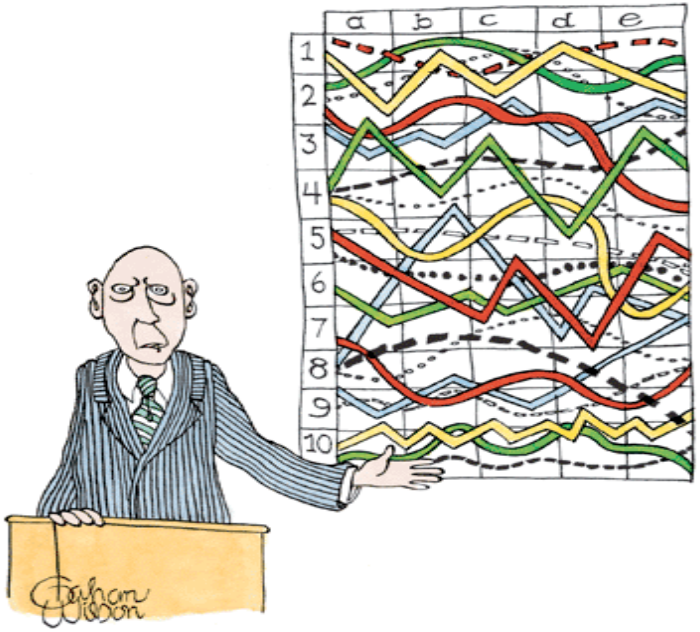


DSF: Calculate apparent K_d for FKBP12 : rapamycin

- Instructor data tested range of rapamycin concentrations
  - 0.1 nM – 20 µM
- Include model equation in Data summary



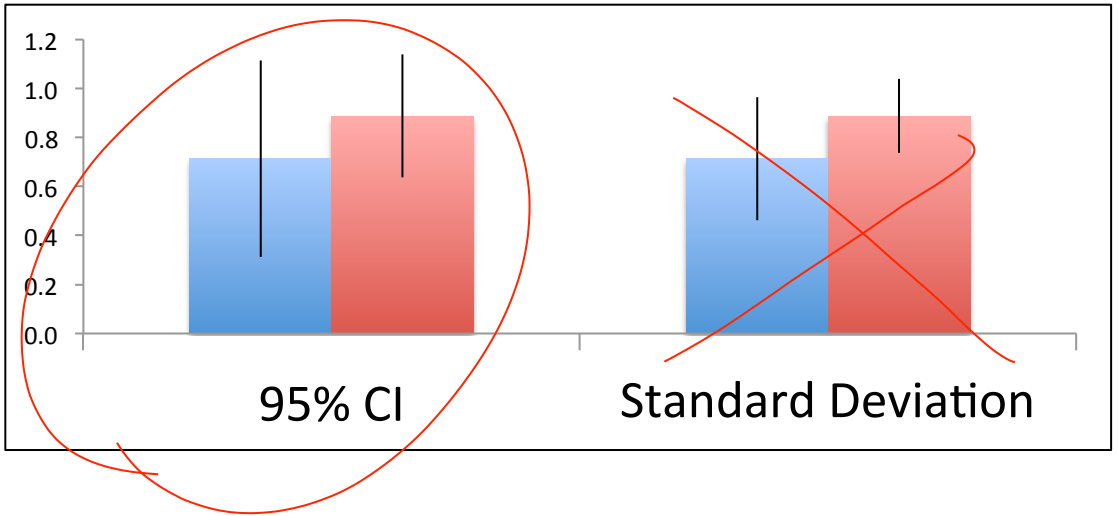
How will you present your results?



“I’ll pause for a moment so you can let this information sink in.”

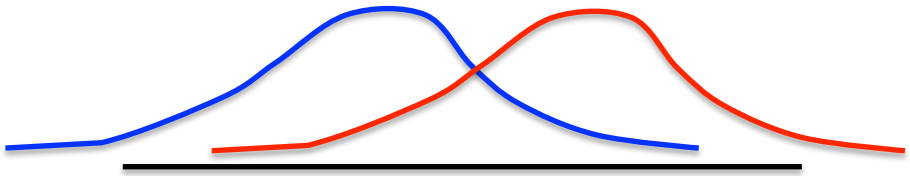
Confidence intervals show the variance in your the data set

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

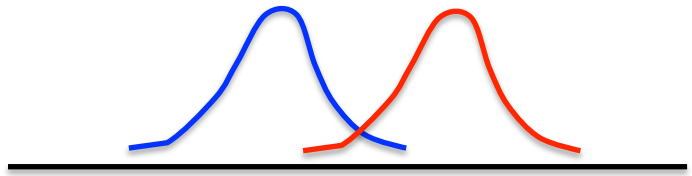


Student's t-test used to determine if populations are significantly different

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



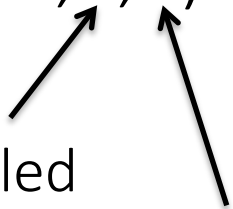
high noise



low noise

Calculating Student's t in excel

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



two-tailed

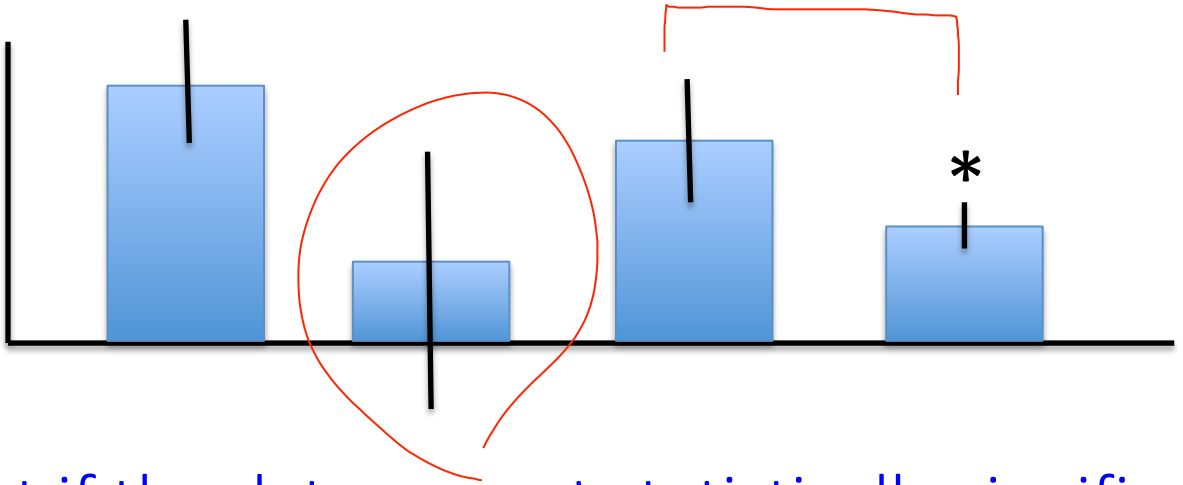
unequal variance

ONLY compare 1 variable at a time.

Can only compare two data sets at a time!

How will you use statistics in your analysis?

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



What if the data are not statistically significant?

Be sure to post your data to the wiki!

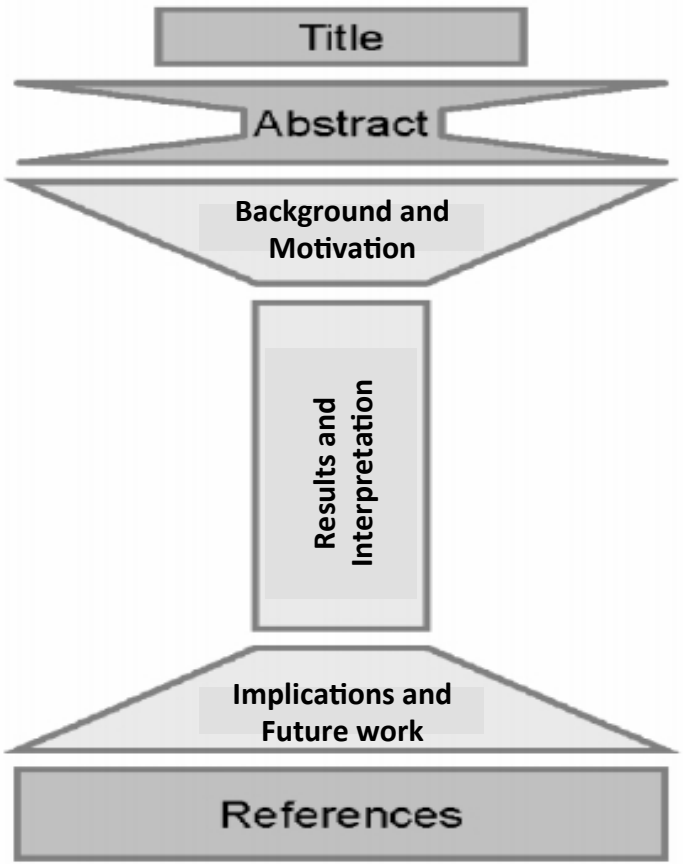
- For the PPIase assay:
  - Single plot with all curves
  - Specific activity calculations
- For the DSF assay:
  - Single plot with all first derivative curves
  - Tm values
- Should be uploaded by 10 pm tonight!

Be sure all information is clearly labeled in excel spreadsheet

Additional notes for Data summary

- Use class data
  - PPIase: compare activity
  - 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!

Structure of scientific writing



Title: take-home message
Abstract: NOT in bullet points!

PUT on Same page

In bullet points:

-Background and Motivation (references)

-Results and Interpretation

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Implications and Future work (references)

References (see wiki for format suggestions)

For today…

- Use class time to analyze data and ask questions
- Post results to Class data tab by 10p tonight!!!

For M2D1…

- Read Mod 2 overview and M2D1 introduction