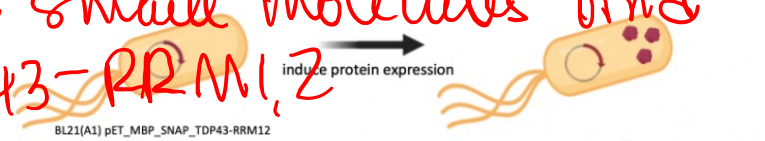
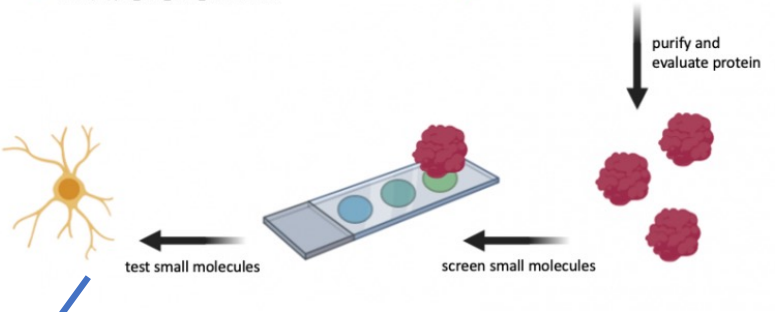
M2D7: Complete CETSA experiment and analyze data

- Prelab discussion
- Analyze CETSA data
- Work on Research Article
- Quiz

Mod2 Experimental Overview

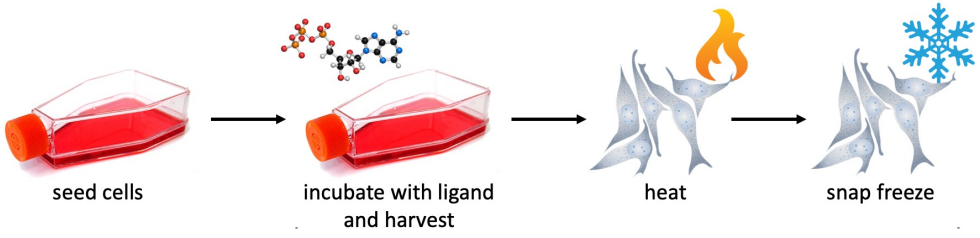
Overview Question: Can we find small molecules bind TDP43-RRM1,2



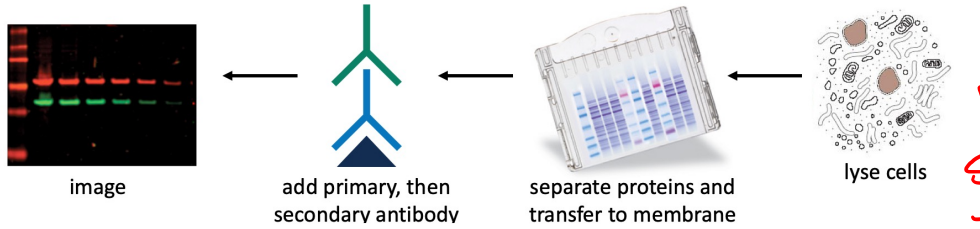


Mod2 Overall

CETSA Overview



M3D1

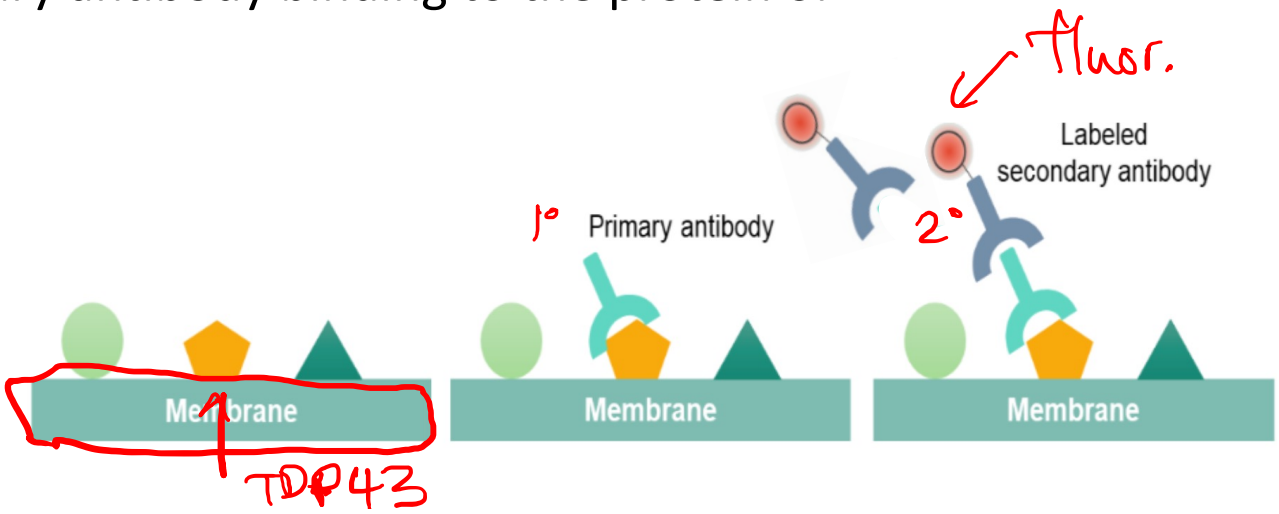


CETSA Research Q: Is there evidence of small molecules binding TDP43 in cells?

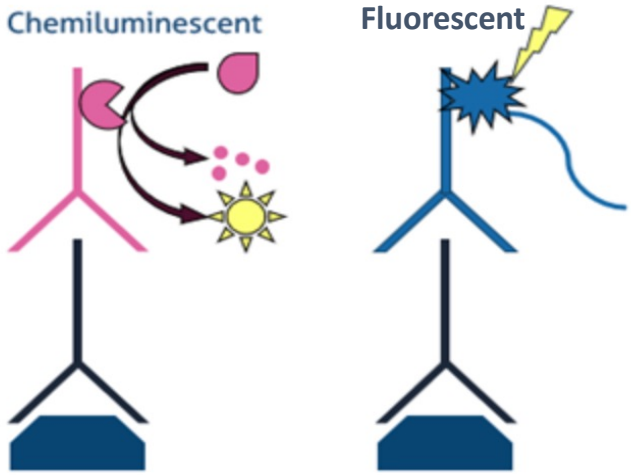
Western blotting

- AKA: immunoblotting
- Uses Primary antibody raised against proteins of interest to identify protein bands on the blot
- Uses Secondary antibody raised against the species of the primary antibody to visualize primary antibody binding to the protein of interest
- Semi-quantitative

Bonus point: Why is it called the Western blot?



Visualizing western blots

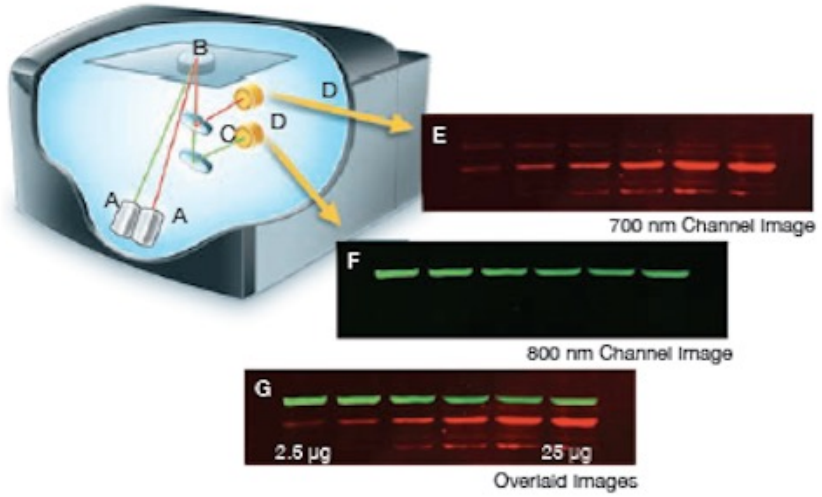


- Once you have antibodies bound to your protein of interest, you need to visualize it

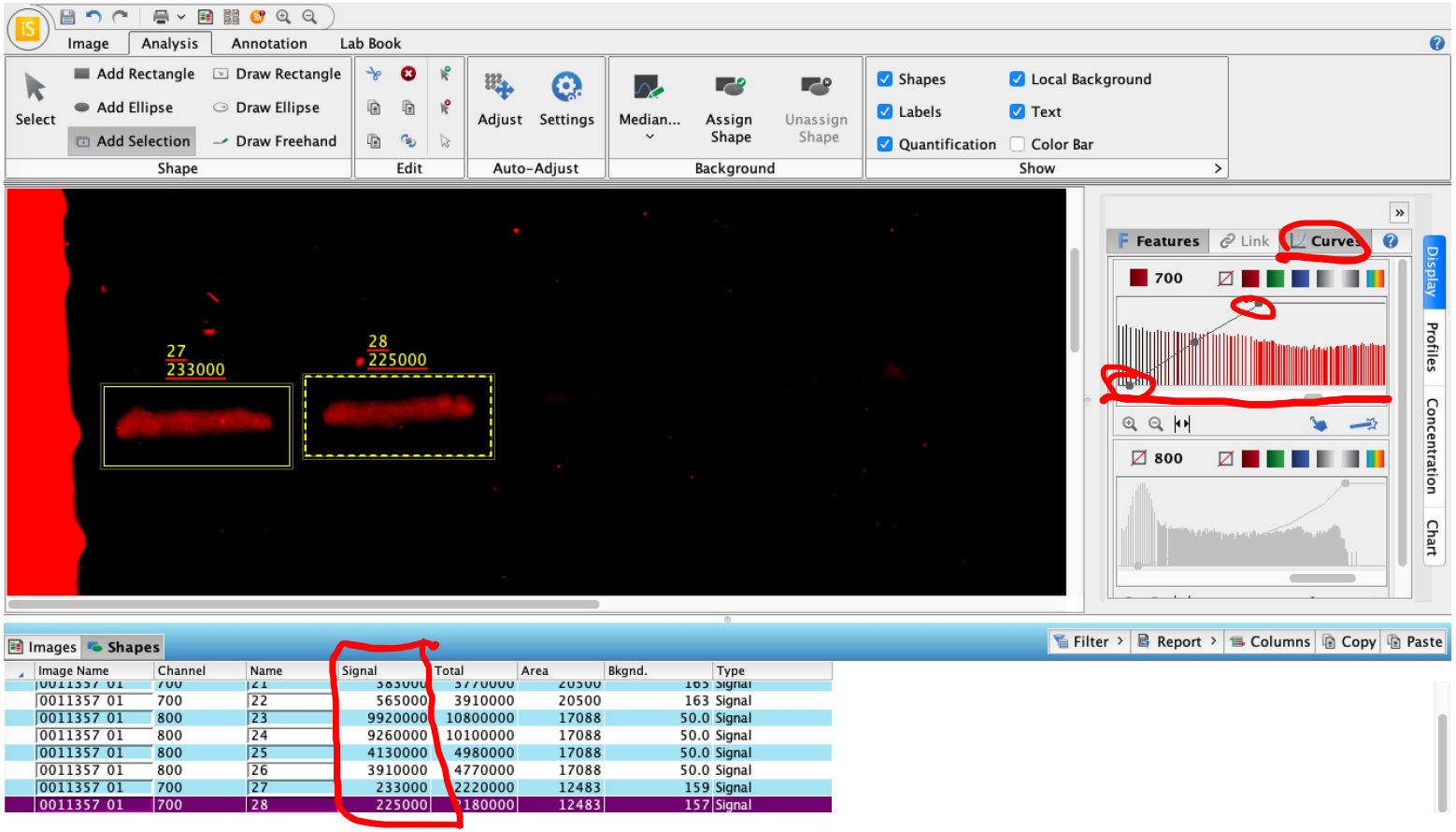
- Most common ways:
  - Chemiluminescence
    - HRP enzyme makes light
    - Film light → stable image on film
  - Fluorescence
    - IR infared fluorophore conj. to secondary
    - Digital scan laser create digital image

Visualizing your Western blot

- Licor imaging system
- Uses infrared conjugated secondary antibodies
- Lasers inside Licor box allows excitation of 700 and 800nm wavelengths
- Produce overlaid image from both channels to identify protein of interest and loading control on the same blot



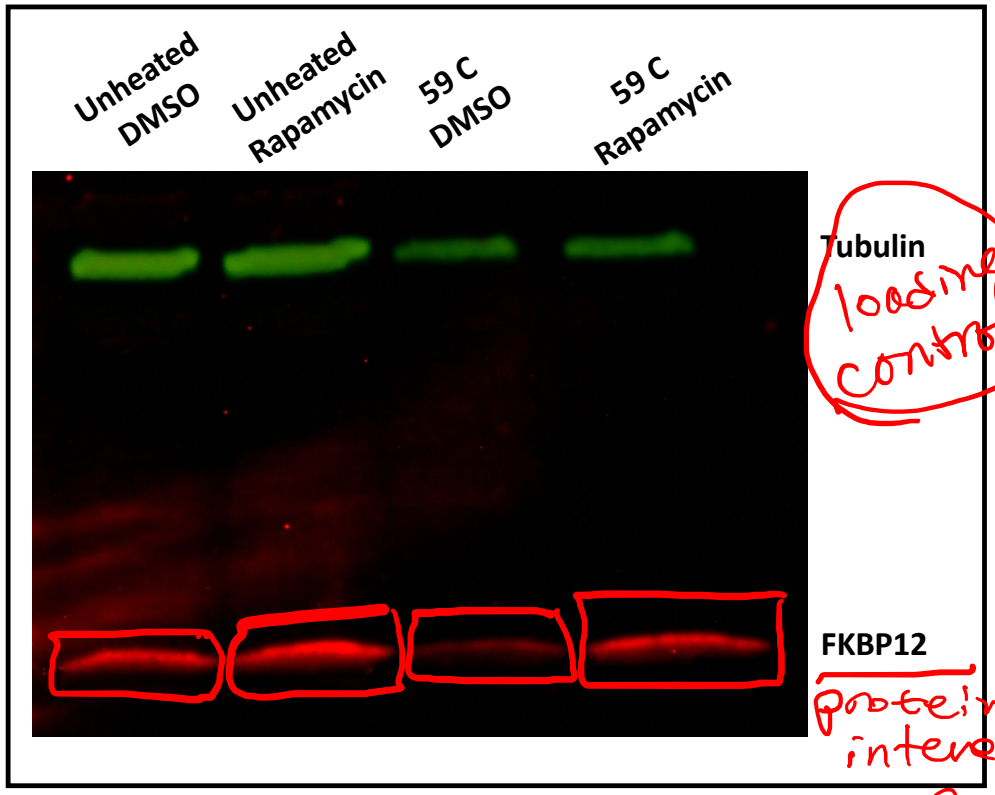
How will we analyze the data?



adjust intensity of fluor image

Example analysis from previous semester data

Raf = small molecule



Data:

	FKBP12 Signal Ratio to Unheated DMSO
Unheated DMSO	1
Unheated Rapamycin	1.305980529
59 C DMSO	0.484005563
59 C Rapamycin	1.207232267

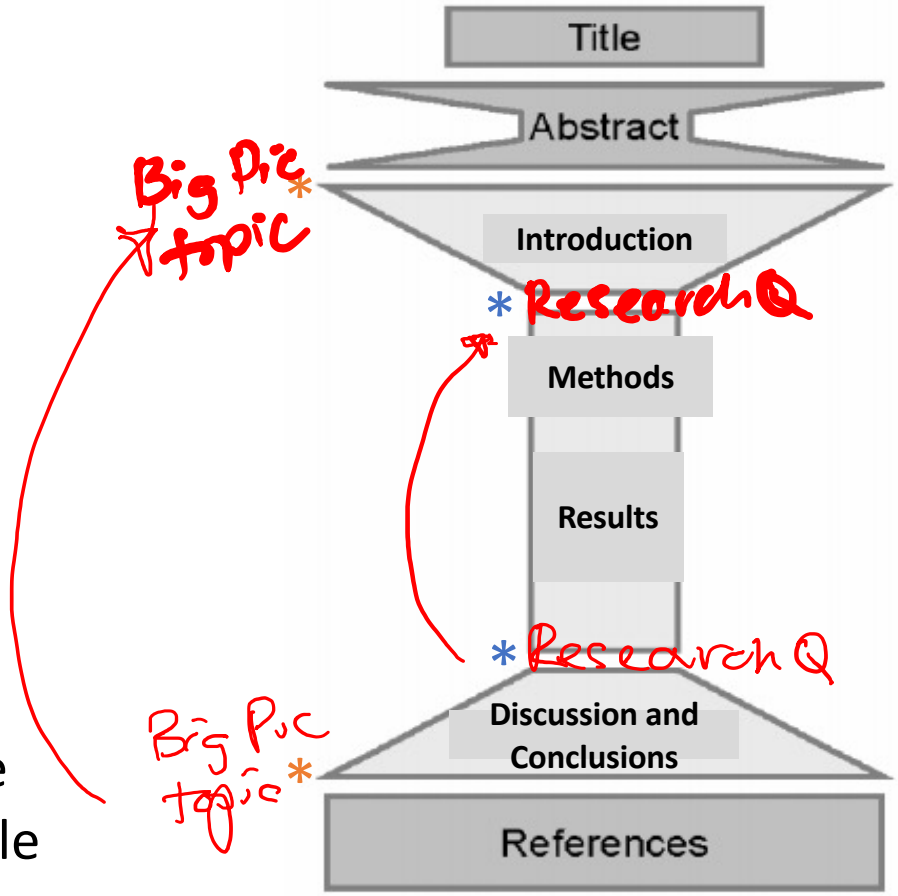
Analysis:

- The heated DMSO treated group shows a 50% loss of FKBP12 signal compared to the unheated DMSO group.
- Rapamycin (small molecule) treatment stabilized the FKBP12 protein so that it maintained unheated levels of expression.
- Tubulin decreased with heat, but there was no apparent effect of Rapamycin on Tubulin stabilization.

Research Article (AKA: Read the Wiki)!

Title/Abstract: 10%
Introduction: 10%
Methods: 20%
Results: 50%
Discussion: 10%

- Write in paragraphs
- Submit a Word Doc OR PDF
- Figures can be incorporated into the Word document OR be a separate file



Research Article: Introduction and Methods

Introduction:

- Tell a cohesive story
  - Use transitions to link ideas together to create the story
  - Don't forget the hourglass narrative!
- Start with "big picture" and zoom in to your specific research question

Methods:

- Revise the draft you've produced
- Add jupyter notebook methods (extra gift on M2D3)
- Add CETSA methods

Cell culture
CETSA
WB

Jupyter notebook methods

The analysis of images to quantify the intensity and position of the 635nm signal associated with putative small molecule binders was performed using Python code integrated in a Jupyter notebook (a gift from Rob Wilson, Koehler Laboratory). The signal-to-noise ratio (SNR) from each compound was used to calculate the robust Z-scores for each compound averaged over replicates, and the “hits” were identified as compounds with Z-score > 5.

Research Article: Results

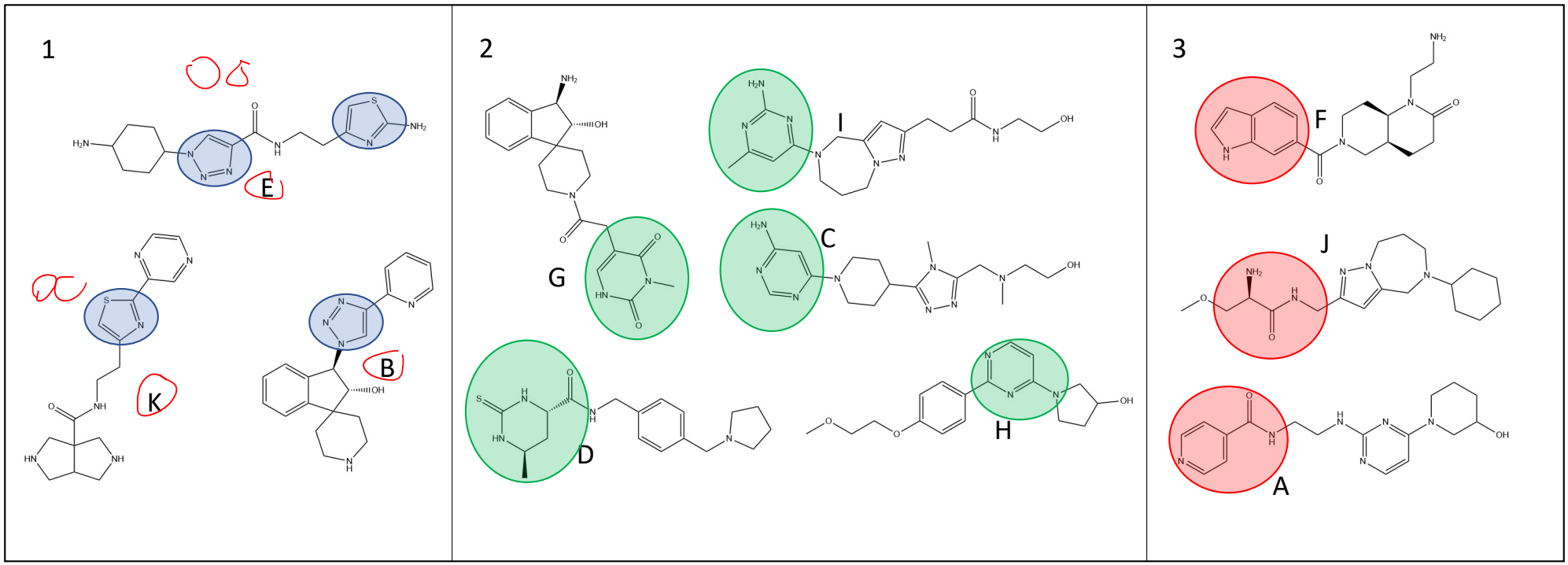
- Make the figure title a take-home message (emphasize the conclusion)
- Use a topic sentence at the beginning of the figure caption

- Introduce the overall goal of the experiment

- Detail the data presented
  - Point out controls
  - Don’t forget to address all data you present
  - Focus on what you see, leave interpretation (what it means) for the discussion

- Transition to the next experiment

Results highlight: Examine binders to identify common structures



- Manuscript writing allows for data interpretation
- Be careful not to overinterpret
  - Reviewers will reject a paper where conclusions aren't considered justified

Research Article: What figures do you want to include?

Schematics

- Overview schematics
- techniques:
  CETSA

Figures / Tables

- SDSPAGE (BCA optional must be in results text)
- SMM (choose your own adventure)
  - hit structures
  - # of hits out of total #
  - SMILES
  - table? [• 2 scores
    • images of spot (one example)
  - CETSA plot, gel images

* use smiles to get compound structure
  Is you come up w label or name

Research Article: Discussion

- **Reiterate the overall goal of the experiment and the major finding(s)**
  address Research Q
- Discuss the data presented
  - What is particularly interesting?
  - What does this data indicate? interpret
  - Propose experiments to address deficiencies in the data/technique
  - **Keep the order of the discussion the same as the results!**
    don't have to discuss everything!
- Transition to future work and overall conclusions (tie back into the big picture)

For Today

- Analyze CETSA data
- Work on Research Article
- Quiz @ 3:30pm

- Notebook to be checked is **M2D1** due Sat @ 10pm

For M3D1

- Read Mod3 overview and M3D1 Introduction

Analyzing CETSA WB with Image Studio

Image Name	Channel	Name	Signal	Total	Area	Bkgnd.	Type
0011361_01	700	29	719000	2820000	11394	184	Signal
0011361_01	700	30	939000	2920000	11394	174	Signal
0011361_01	700	31	348000	2180000	11394	161	Signal
0011361_01	700	33	868000	2700000	11394	161	Signal

- Image Name: Name of entire image in Image Studio
- Channel: Wavelength of signal detection (700 or 800)
- Name: Number assigned to rectangle drawn around a band. Each rectangle for each channel will have a unique number
- Total: Sum of individual pixel intensities in the rectangle
- Area: Total number of pixels enclosed by the rectangle
- Bkgnd: Value assigned for background subtraction (default= mean pixel intensity of background)
- Type: What being measured (i.e. signal or background). More relevant for manually determining background.
- Signal: Sum of the pixel intensity values in the rectangle minus the product of the background and area

Signal = Total – (Background x Area)

Assessing signal quality of the protein bands