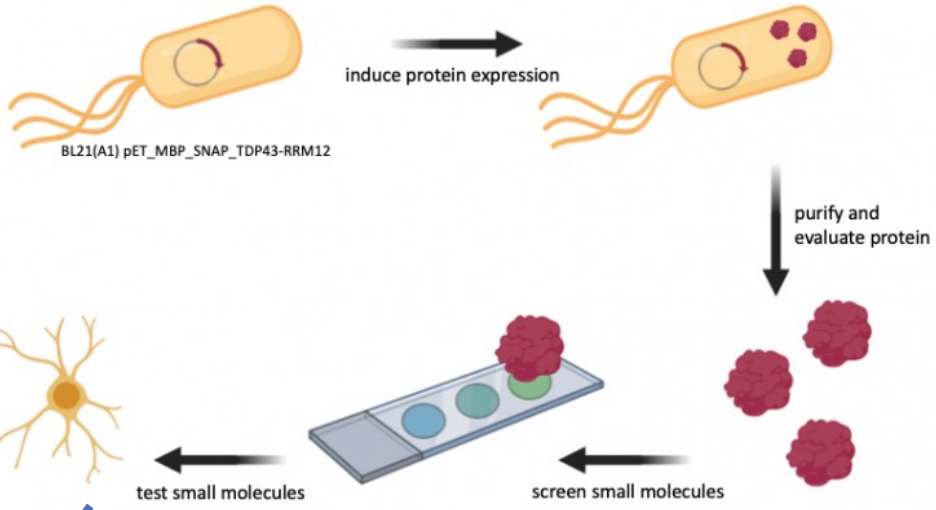
# M2D7: Complete CETSA experiment and analyze data

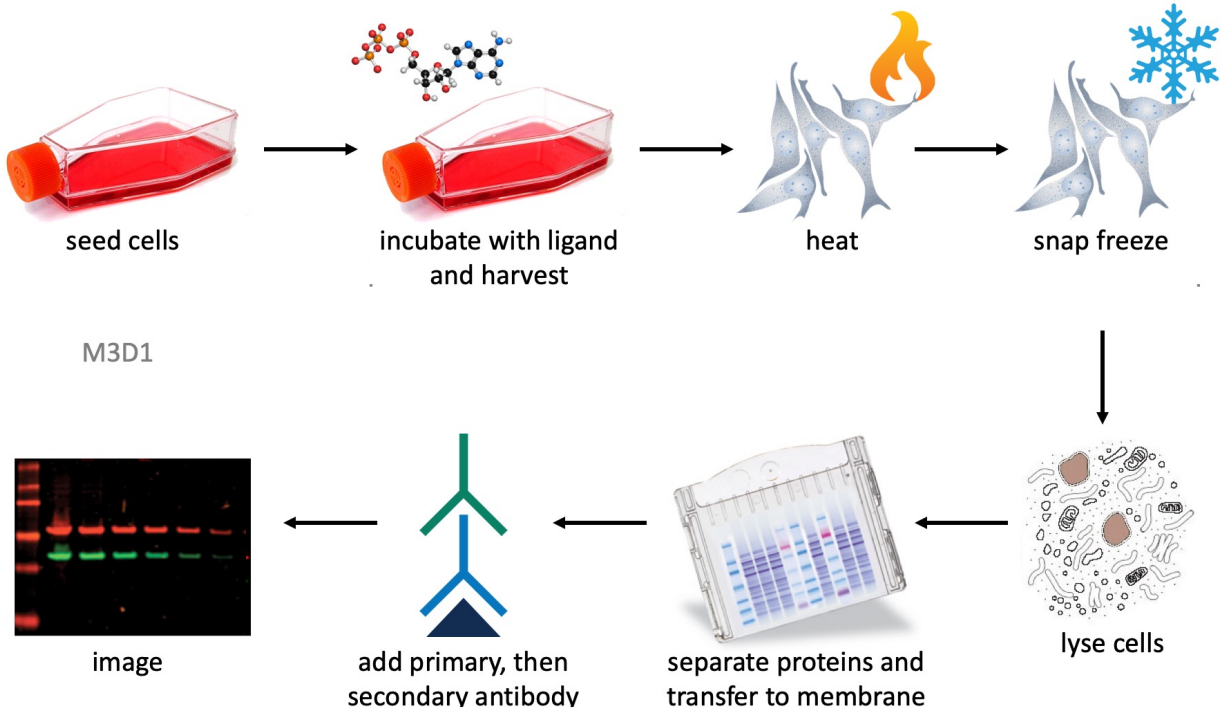
- Prelab discussion
- Analyze CETSA data
- Work on Research Article
- Quiz

# Mod2 Experimental Overview




**Mod2 Overall**

## CETSA Overview

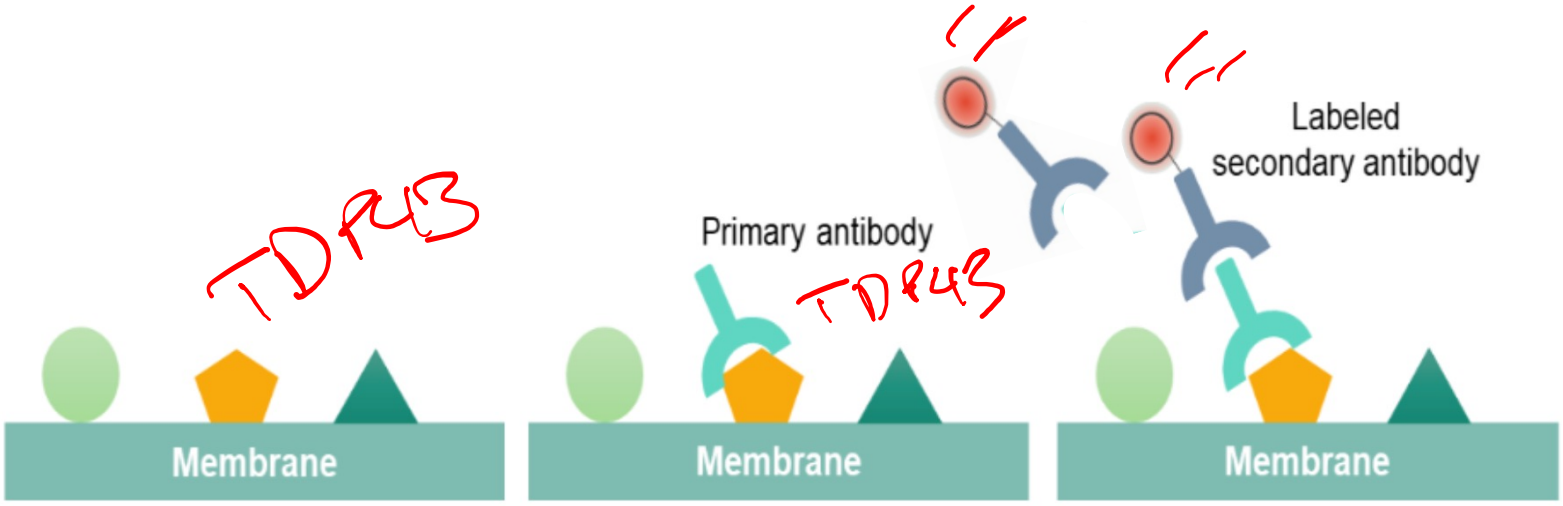



M3D1

# 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

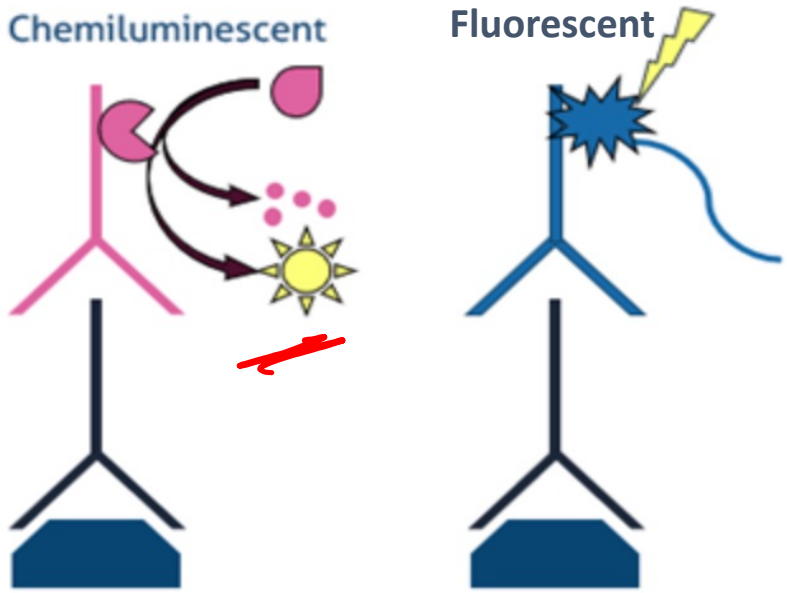
Bonus point: Why is it called the Western blot?



# Visualizing western blots

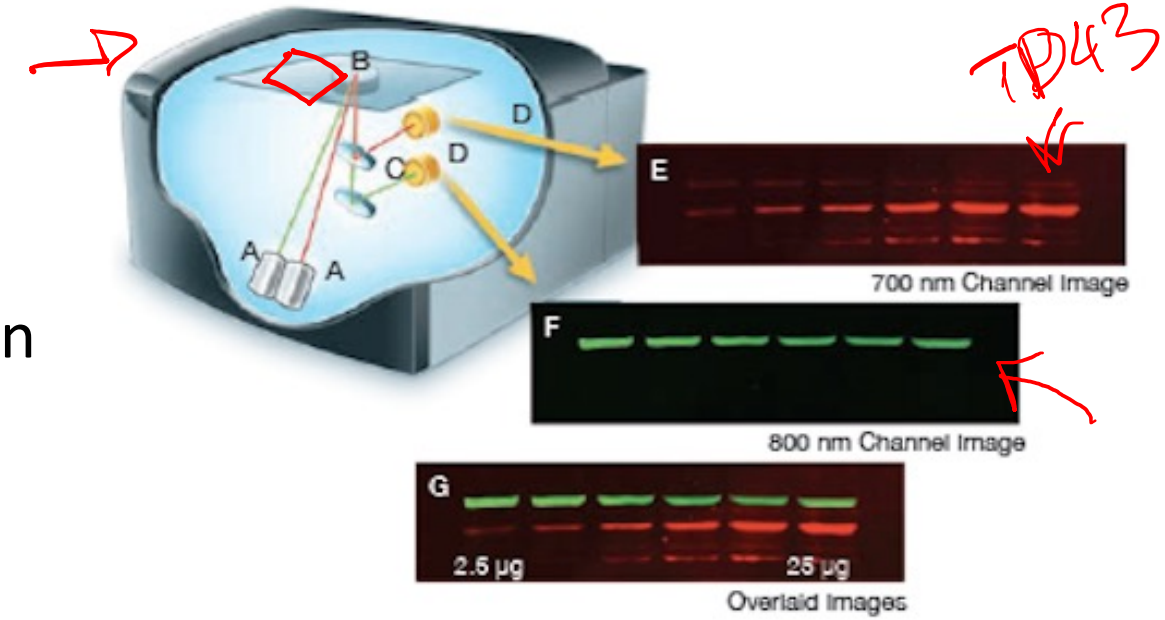
- Once you have antibodies bound to your protein of interest, you need to visualize it

- Most common ways:
  - Chemiluminescence
    - HRP
    - Film
  - Fluorescence
    - IR - Infared 2° Ab
    - Digital scan

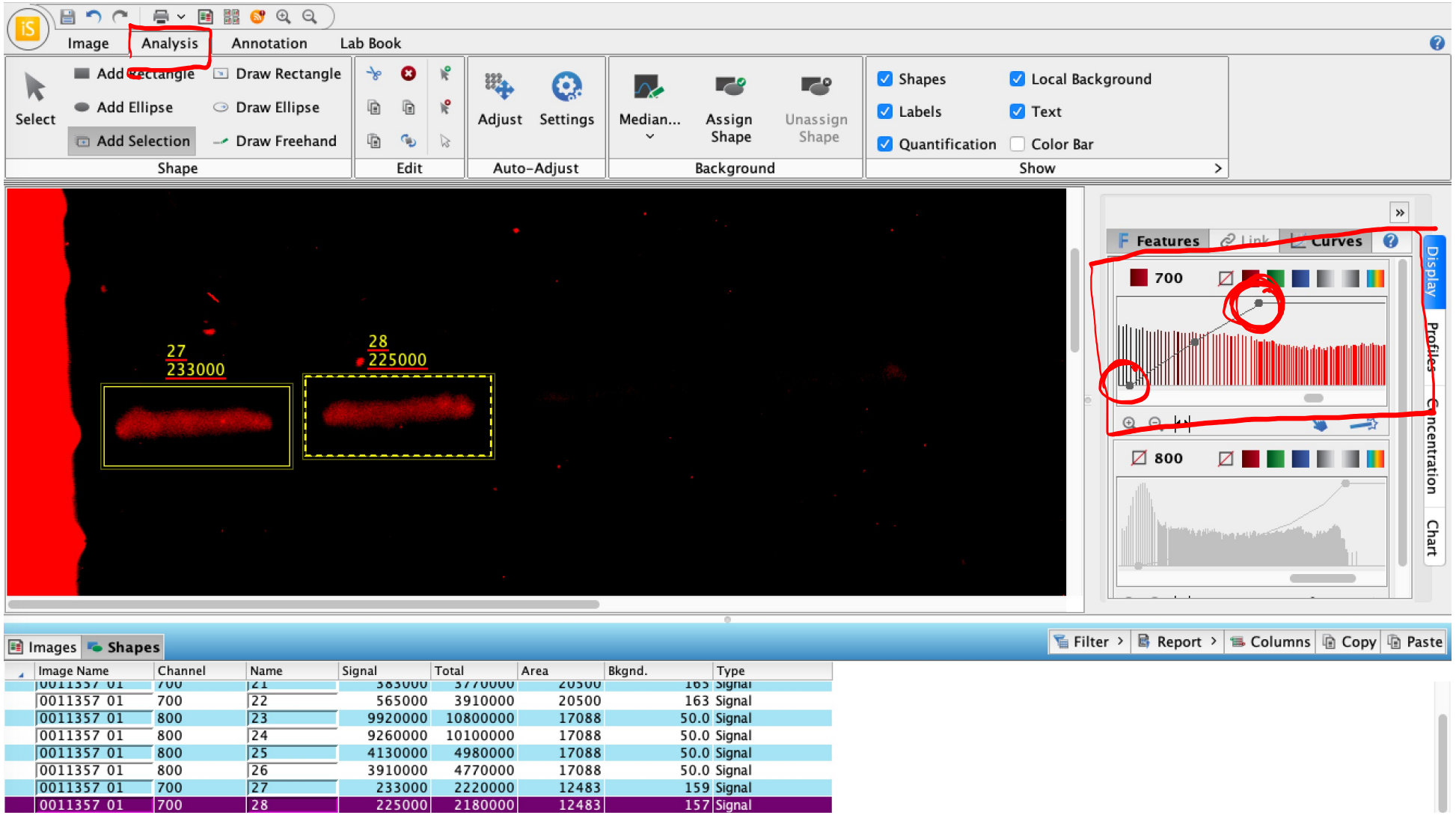


# Visualizing your Western blot

- Licor 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?




| Image Name | Channel | Name | Signal | Total | Area | Bkgnd. | Type |
|---|---|---|---|---|---|---|---|
| 0011357 01 | 700 | 21 | 383000 | 3770000 | 20500 | 163 | Signal |
| 0011357 01 | 700 | 22 | 565000 | 3910000 | 20500 | 163 | Signal |
| 0011357 01 | 800 | 23 | 9920000 | 10800000 | 17088 | 50.0 | Signal |
| 0011357 01 | 800 | 24 | 9260000 | 10100000 | 17088 | 50.0 | Signal |
| 0011357 01 | 800 | 25 | 4130000 | 4980000 | 17088 | 50.0 | Signal |
| 0011357 01 | 800 | 26 | 3910000 | 4770000 | 17088 | 50.0 | Signal |
| 0011357 01 | 700 | 27 | 233000 | 2220000 | 12483 | 159 | Signal |
| 0011357 01 | 700 | 28 | 225000 | 2180000 | 12483 | 157 | Signal |

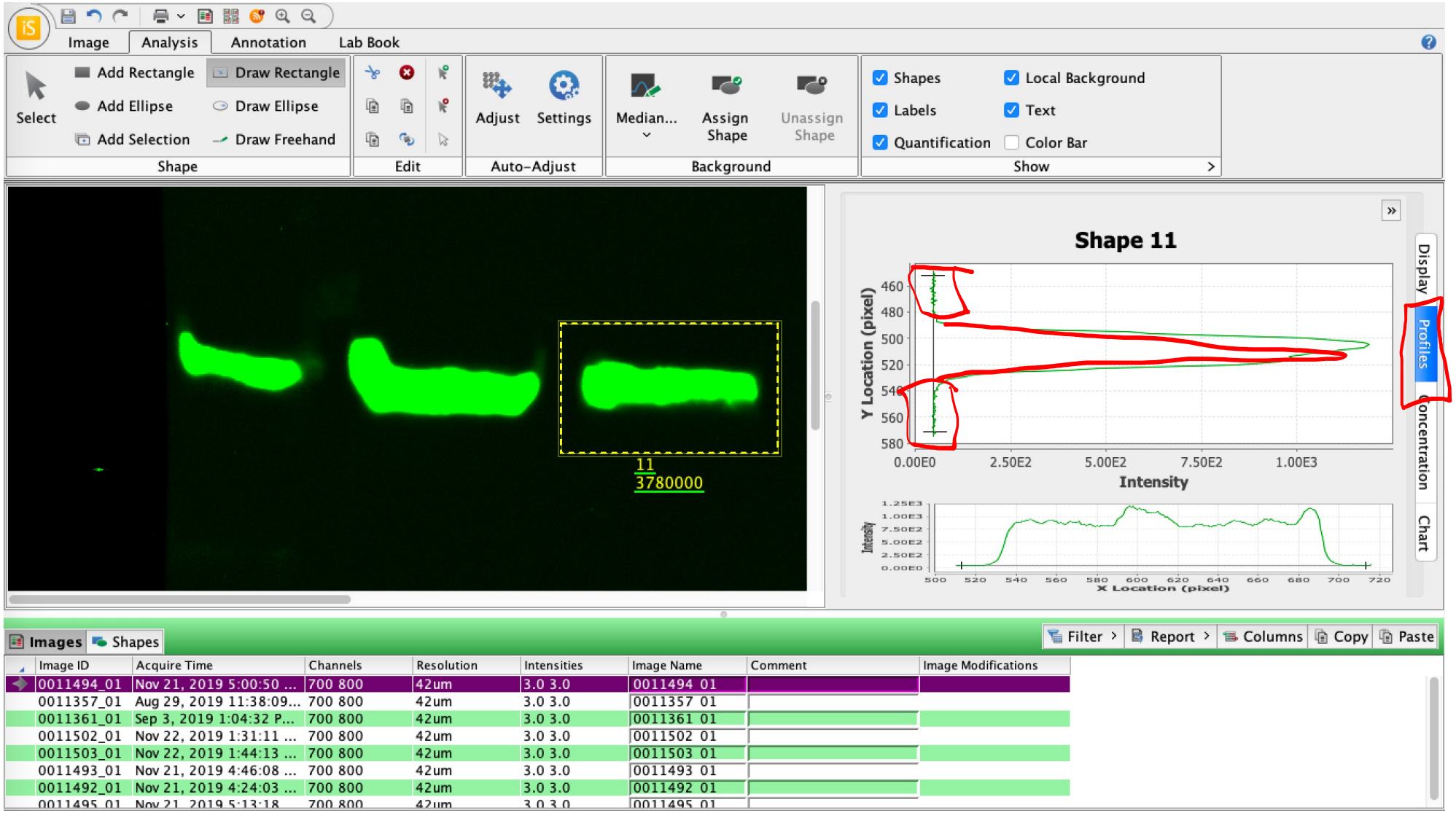
# Analyzing CETSA WB with Image Studio

| 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 |

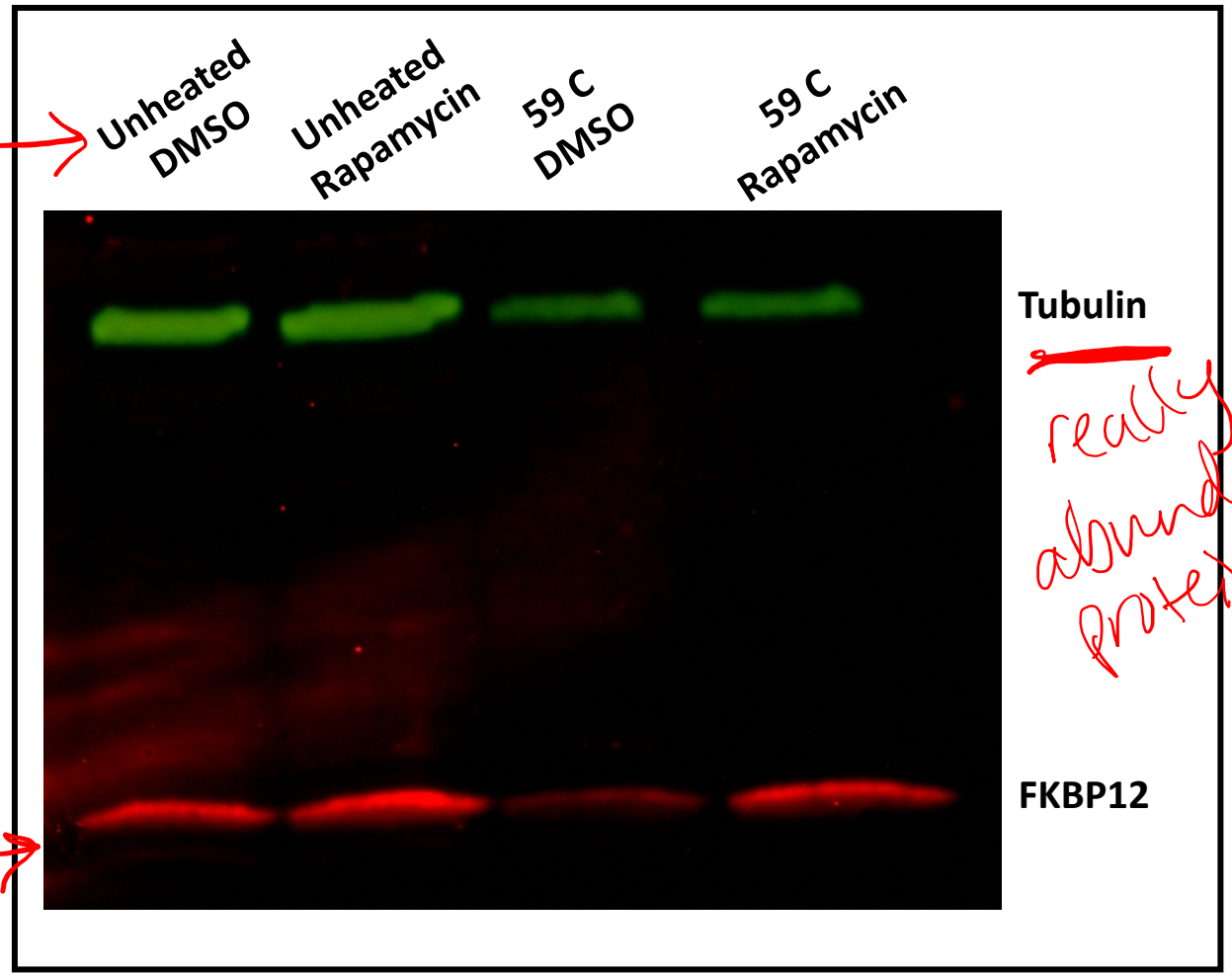
- Image Name: Name of entire image in Image Studio
- Channel: Wavelength of signal detection (700 or 800)
- Name: Number assigned to rectangle drawn around a band. Each rectangle for each channel will have a unique number
- Total: Sum of individual pixel intensities in the rectangle
- Area: Total number 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



# Example analysis from previous semester data




Data:

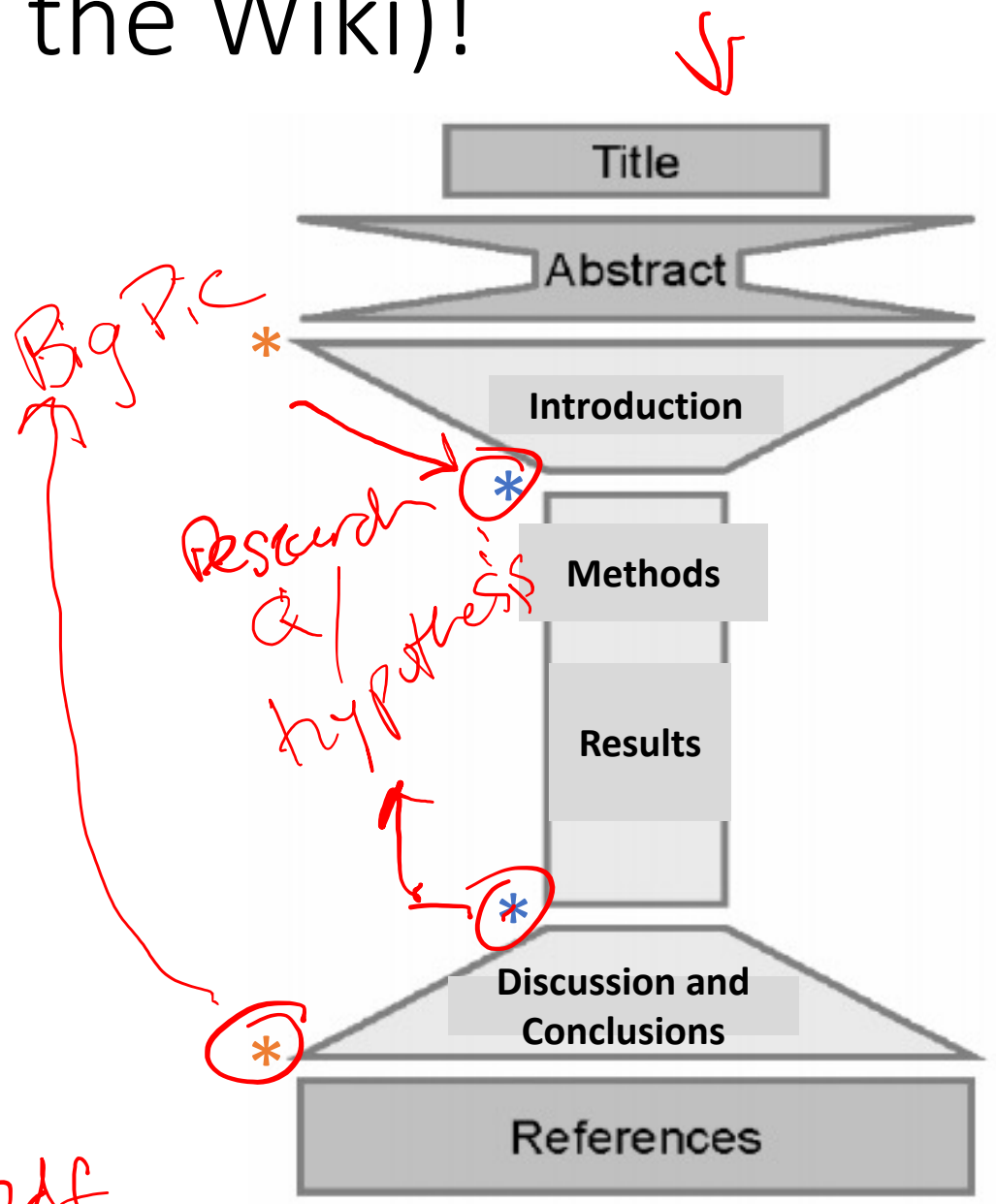
| | FKBP12 Signal Ratio to Unheated DMSO |
|---|---|
| Unheated DMSO | 1 |
| Unheated Rapamycin | 1.305980529 |
| 59 C DMSO | 0.484005563 |
| 59 C Rapamycin | 1.207232267 |

Analysis:

- The heated DMSO treated group shows a 50% loss of FKBP12 signal compared to the unheated DMSO group.
- Rapamycin 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

# 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

# Research Article: What figures do you want to include?

Schematics

- protein purification

SMM Results

- table of compounds/ binders
- sample of SMM sputs
  - SMILES
  - Z score

- SDS Page
  - don't have to show BCA BUT
  - must discuss BCA data
- Compound structures
- CETSA results

# Research Article: Discussion

- **Reiterate the overall goal of the experiment and the major finding(s)**

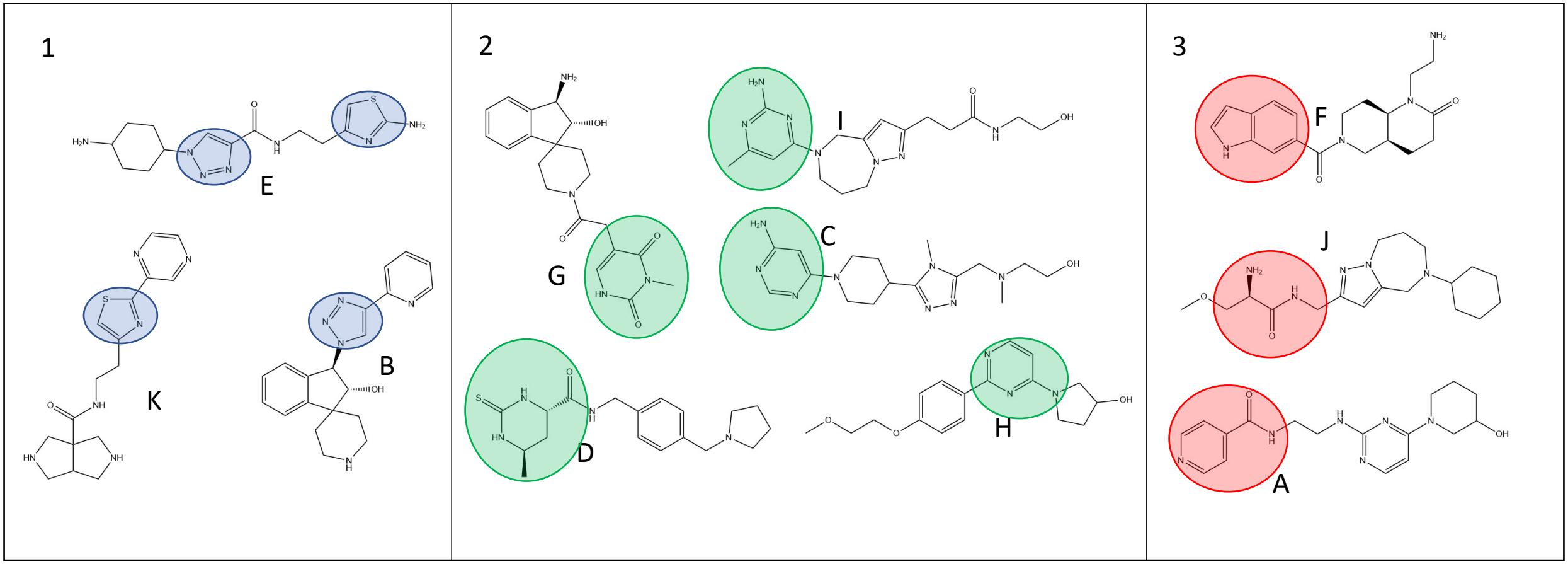
- Discuss the data presented
  - What is particularly interesting?
  - What does this data indicate?

- Propose experiments to address deficiencies in the data/technique

- Keep the order of the discussion the same as the results

- Transition to future work and overall conclusions (tie back into the big picture)

# 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

# For Today

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

- Notebook to be checked is **M2D1**

# For M3D1

- Read Mod3 overview and M3D1 Introduction