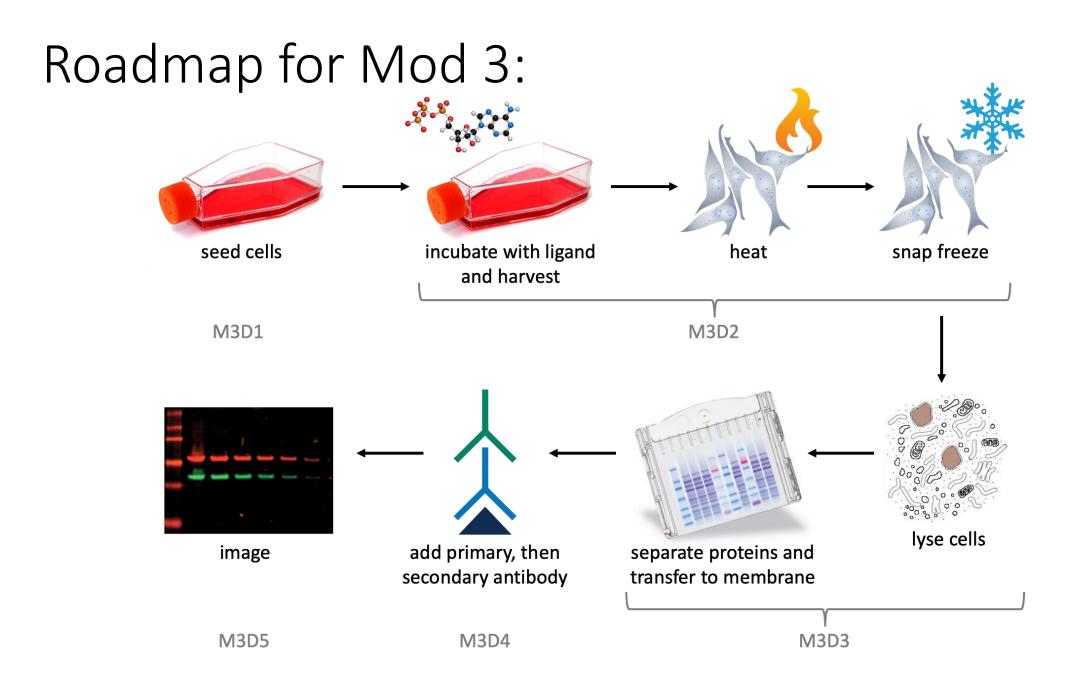


- 1. Last quiz!
- 2. Prelab discussion
- 3. Analyze CETSA results
- 4. Prepare Mini-report



Notebook untry due: M3D4



#### How will we analyze the data?

|        | Image Analysis Annotation Lal   Add Rectangle Draw Rectangle | Book         | ₩ 333   | + O           |          |                         | 🗸 Shapes 💽       | 💋 Local Back      | ground   |  |
|--------|--|--------------|---------|---------------|----------|-------------------------|------------------|-------------------|--|--|
| Select |  |              | 😤 🛛 Adj | just Settings | Median A | ssign Unassign          | 🗹 Labels 🛛 🔽     | 🖌 Text            |  |  |
|        | Add Selection - Draw Freehand<br>Shape                       | Edit         |         | Auto-Adjust   |          | Shape Shape<br>ckground | ✓ Quantification | Color Bar<br>Show | >  |  |
|        |  | 28<br>225000 |         | •             |          |                         |                  |                   | F Features   ∅     700   ∅     0   ∅     0   ♀     0   ♀     1   800     1   1 |  |

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| E | = | mages - snape: | 5       |      |         |          |       |        |        |
|---|---|----------------|---------|------|---------|----------|-------|--------|--------|
|   |   | Image Name     | Channel | Name | Signal  | Total    | Area  | Bkgnd. | Туре   |
|   |   | 10011321 01    | 700     | 121  | 383000  | 3770000  | 20500 | 102    | Signai |
|   |   | 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 |

💽 Images 📪 Shan

# Analyzing CETSA (WB) with Image Studio

| Image Name | Channel | Name | Signal | Total   | Area  | Bkgnd. | Туре   |
|------------|---------|------|--------|---------|-------|--------|--------|
| 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 |

- <u>Image Name</u>: Name of entire image in Image Studio
- <u>Channel</u>: Wavelength of signal detection (700 or 800)
- <u>Name</u>: Number assigned to rectangle drawn around a band. Each rectangle for each channel will have a unique number
- <u>Total</u>: Sum of individual pixel intensities in the rectangle
- <u>Area</u>: Total number of pixels enclosed by the rectangle
- <u>Bkgnd</u>: Value assigned for background subtraction (default= mean pixel intensity of background)
- <u>Type</u>: 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

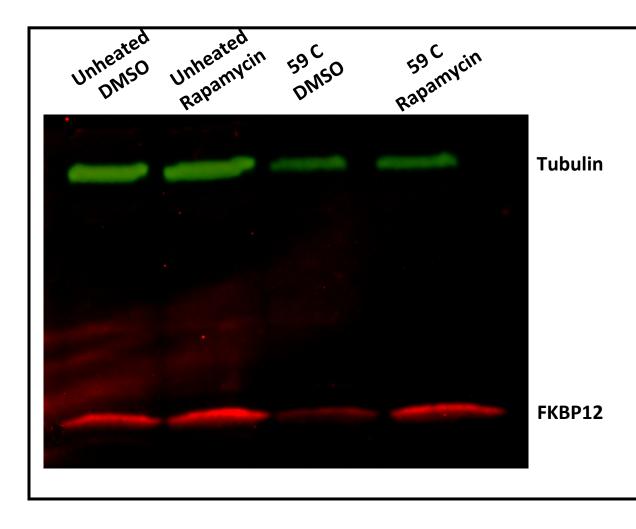
#### Signal = Total – (Background x Area)

### Assessing signal quality of the protein bands

| (IS)   |  | h Da ali |                 |                       |                            |  |
|--------|--|----------|-----------------|-----------------------|----------------------------|--|
| Select | Image Analysis Annotation La   Add Rectangle Draw Rectangle   Add Ellipse Draw Ellipse   Add Selection Draw Freehand | b Book   | Adjust Settings | Median Assign Una     | <b>e</b><br>Issign<br>hape | ✓ Shapes   ✓ Local Background     ✓ Labels   ✓ Text     ✓ Quantification   Color Bar   |
|        | Shape  | Edit     | Auto-Adjust     | Background            |                            | Show >   |
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|----------|------------|-----------------------|----------|------------|-------------|------------|---------|---------------------|------------|------------|-----------|--------|---------|
|          | Image ID   | Acquire Time          | Channels | Resolution | Intensities | Image Name | Comment | Image Modifications |            |            |           |        |         |
|          | 0011494_01 | Nov 21, 2019 5:00:50  | 700 800  | 42um       | 3.0 3.0     | 0011494 01 |         |                     |            |            |           |        |         |
|          | 0011357_01 | Aug 29, 2019 11:38:09 | 700 800  | 42um       | 3.0 3.0     | 0011357 01 |         | -                   |            |            |           |        |         |
|          | 0011361_01 | Sep 3, 2019 1:04:32 P | 700 800  | 42um       | 3.0 3.0     | 0011361 01 |         |                     |            |            |           |        |         |
|          | 0011502_01 | Nov 22, 2019 1:31:11  | 700 800  | 42um       | 3.0 3.0     | 0011502 01 |         | -                   |            |            |           |        |         |
|          | 0011503_01 | Nov 22, 2019 1:44:13  | 700 800  | 42um       | 3.0 3.0     | 0011503 01 |         |                     |            |            |           |        |         |
|          | 0011493_01 | Nov 21, 2019 4:46:08  | 700 800  | 42um       | 3.0 3.0     | 0011493 01 |         | -                   |            |            |           |        |         |
|          | 0011492_01 | Nov 21, 2019 4:24:03  | 700 800  | 42um       | 3.0 3.0     | 0011492 01 |         |                     |            |            |           |        |         |
|          | 0011495 01 | Nov 21 2019 5-13-18   | 700 800  | 42um       | 3030        | 0011495 01 |         |                     |            |            |           |        |         |

#### Example analysis from pilot 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.

# Mini-Report details

- Introduce your investigation (1-2 paragraphs)
- Represent your data in figures / tables / text
  - 1. Ligand structure (figure)
  - 2. Western blot (figure)
  - 3. Analysis of Western blots bands (figure or text)
  - 4. Comparison of ligand  $\mathbf{x}_{m}$  values (table)
- Evaluate the data
  - Does it match the DSF?
  - What technical changes would you make to improve on the preliminary experiment?
  - What are the next steps for this project?

# Important Mod 3 dates

- Research proposal presentation due Thursday, Dec 5 by 1 pm
  - Completed in teams!
  - 12 minute presentation, submitted to Stellar
- Blog post due Friday, Dec 6 by 10 pm
- Mini-report due Monday, Dec 9 by 10 pm
  - Completed in teams!
  - 3 page word document, submitted to Stellar
- Feedback lunch on Tuesday, Dec 10 at 11 am!