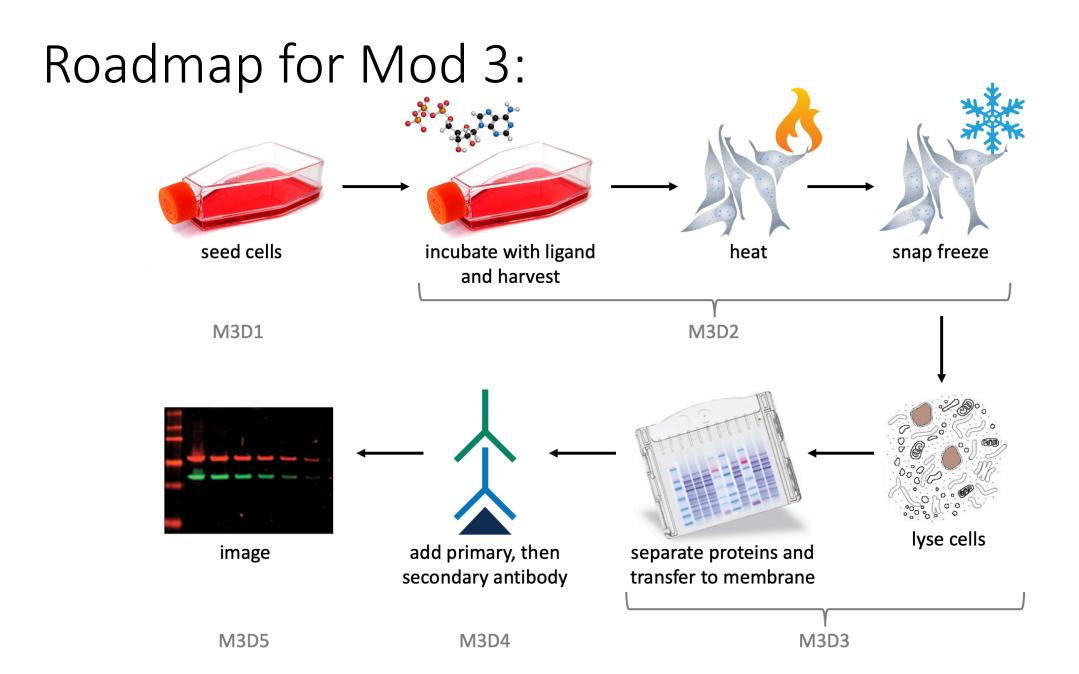


- 1. Last quiz!
- 2. Prelab discussion
- 3. Analyze CETSA results
- 4. Prepare Mini-report



Notebook untry due: M3D4



How will we analyze the data?

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		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

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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

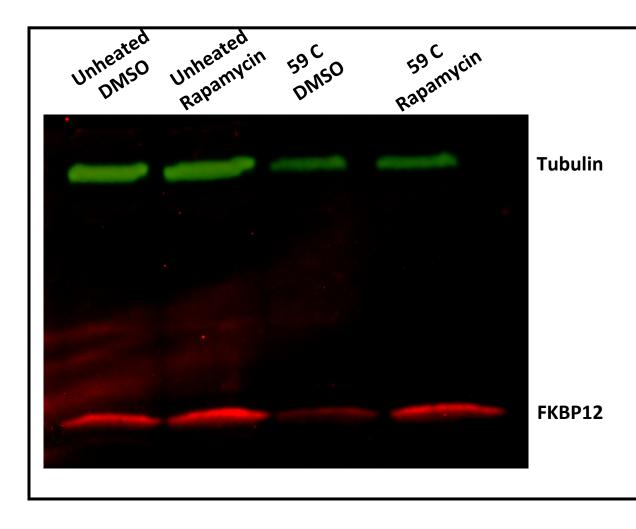
Signal = Total – (Background x Area)

Assessing signal quality of the protein bands

(IS)		h Da ali				
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	Image ID	Acquire Time	Channels	Resolution	Intensities	Image Name	Comment	Image Modifications					
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	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!