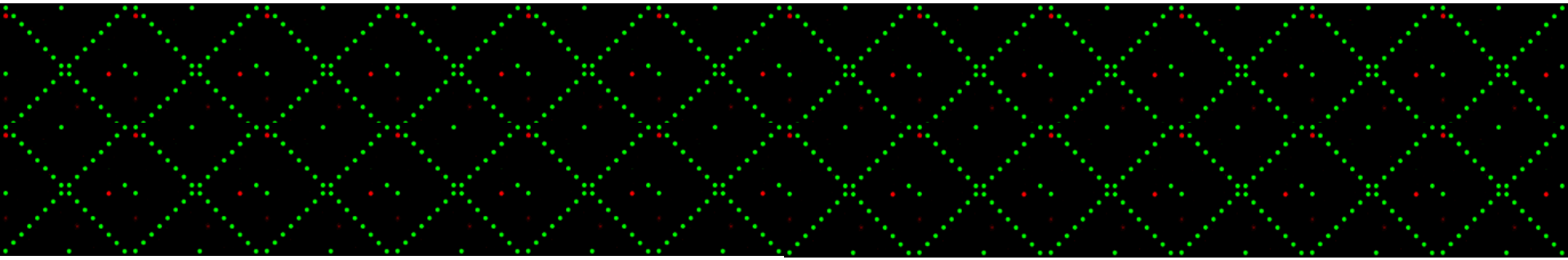
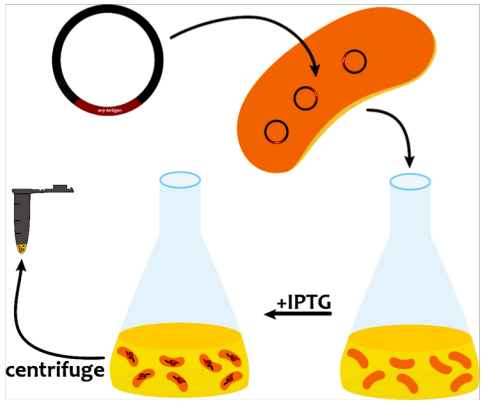


Module 1 wrap up discussion

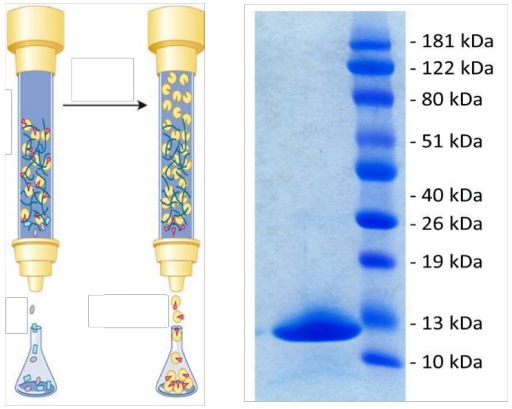
March 5, 2019

Our path to evaluate FKBP12 ligands



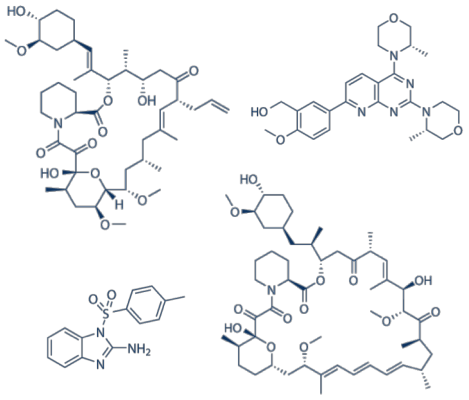
in silico cloning; overexpress FKBP12

lab day 1



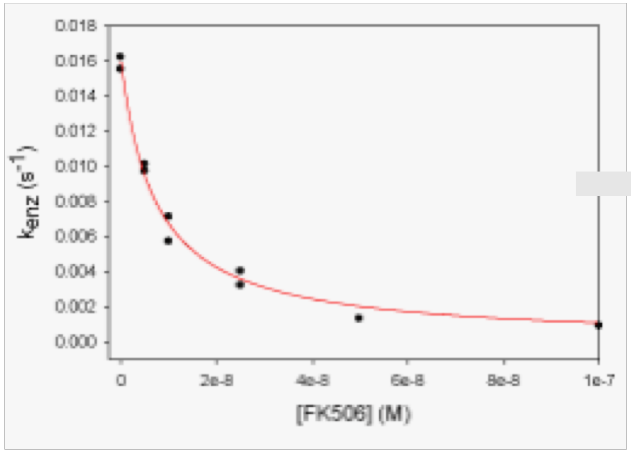
purify and analyze FKBP12

lab days 2 and 4



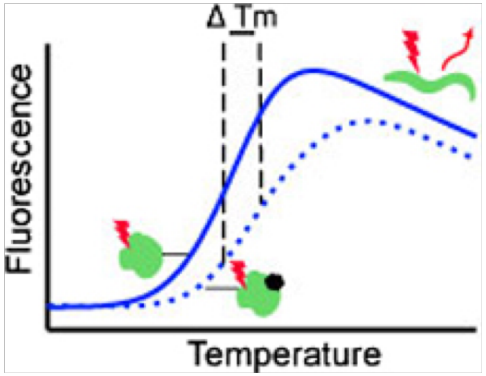
re-analyze screen data

lab day 3



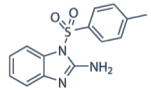
test FKBP12 and ligands in enzyme activity assay

lab day 5



test ligands in FKBP12 binding assay

lab day 6



IC_{50} = X μM

ΔT = Y °C

complete data analysis prioritize best ligands

lab day 7

Report for Module 1

scientific abstract

nature
chemical biology

A small molecule that binds Hedgehog and blocks its signaling in human cells

Benjamin Z Stanton[1,2,7], Lee F Peng[1–3,7], Nicole Maloof[1], Kazuo Nakai[2], Xiang Wang[1], Jay L Duffner[1], Kennedy M Taveras[1], Joel M Hyman[4], Sam W Lee[5], Angela N Koehler[1], James K Chen[4], Julia L Fox[6], Anna Mandinova[5] & Stuart L Schreiber[1,2]

Small-molecule inhibition of extracellular proteins that activate membrane receptors has proven to be extremely challenging. Diversity-oriented synthesis and small-molecule microarrays enabled the discovery of robotnikinin, a small molecule that binds the extracellular Sonic hedgehog (Shh) protein and blocks Shh signaling in cell lines, human primary keratinocytes and a synthetic model of human skin. Shh pathway activity is rescued by small-molecule agonists of Smoothened, which functions immediately downstream of the Shh receptor Patched.

Report for Module 1

project summary

thorough summary of your data and figures with supporting text –

include context so that a scientifically literate reader can understand the work and its broader implications

details related to the format and content are on the 20.109 wiki (example posted)

Report for Module 1

format and content

Layout: Portrait, not landscape.
Font: Arial 14pt for text; Arial 12pt for figure captions.
Text should be written as **bullet points**, not full sentences and paragraphs.

Content details

First page: Title and **Author** information (section/color/names)
Second page: Abstract
Body: 8-12 pages (not including Title and Abstract pages). Recommended section lengths (including both text and figures):
Background and Motivation: 2 slides

Contents of Background and Motivation: The majority of this section will be bulleted text. Include schematic figures when appropriate.

Results and Interpretation: 5-8 slides

Contents of a Results and Interpretation slide: Top half: figure(s) with caption(s). Bottom half: bullet points that present and interpret the data. (Remember that captions should not contain interpretation.)

Figure presentation: In published research figures are rarely a full page in size; rather each plot is usually only 3 inches x 3 inches.

Present you Results and Interpretation such that the figure, caption, and interpretation bullet points **all fit on a single slide**. Remember that when you shrink a figure, you must make sure it remains legible.

Implications and Future Work: 1-2 slides
Contents of Implications and Future Work: This section will be bulleted text.

Background and motivation

suggested topics or figures

Topic: Introduce how novel chemical probes for FKBP12 would enable biological engineering research

Topic: Introduce and discuss the utility of small-molecule microarrays (SMMs) to find putative ligands

Topic: Describe methodologies to evaluate putative ligands via FKBP12 binding and activity assays

Figure: Simplified schematic of '*Critical Path for Probe Discovery and Characterization*'

Topic: Discuss your experimental goal

Schematic: Experimental approach

Results and Interpretation

suggested topics or figures

Protein purification

Schematic: Experimental design

Topic: FKBP12 purification

Figure: Image of polyacrylamide gel

Figure: Graph or table displaying cell protein concentration

Ligand characterization

Schematic: Experimental approach

Topic: Identification of positive hits from Spring 20.109 SMM data

Figure: Chemical structures for compounds tested

Results and Interpretation

suggested topics or figures

PPI'ase enzymatic assay

Schematic: Experimental design

Topic: Explain the enzymatic reaction that you evaluated (from Wiki)

Figure: Specific Activity calculation for your FKBP12

Figure: Activity plots for each condition tested: your FKBP12, Abcam FKBP12, different ligands, DMSO control

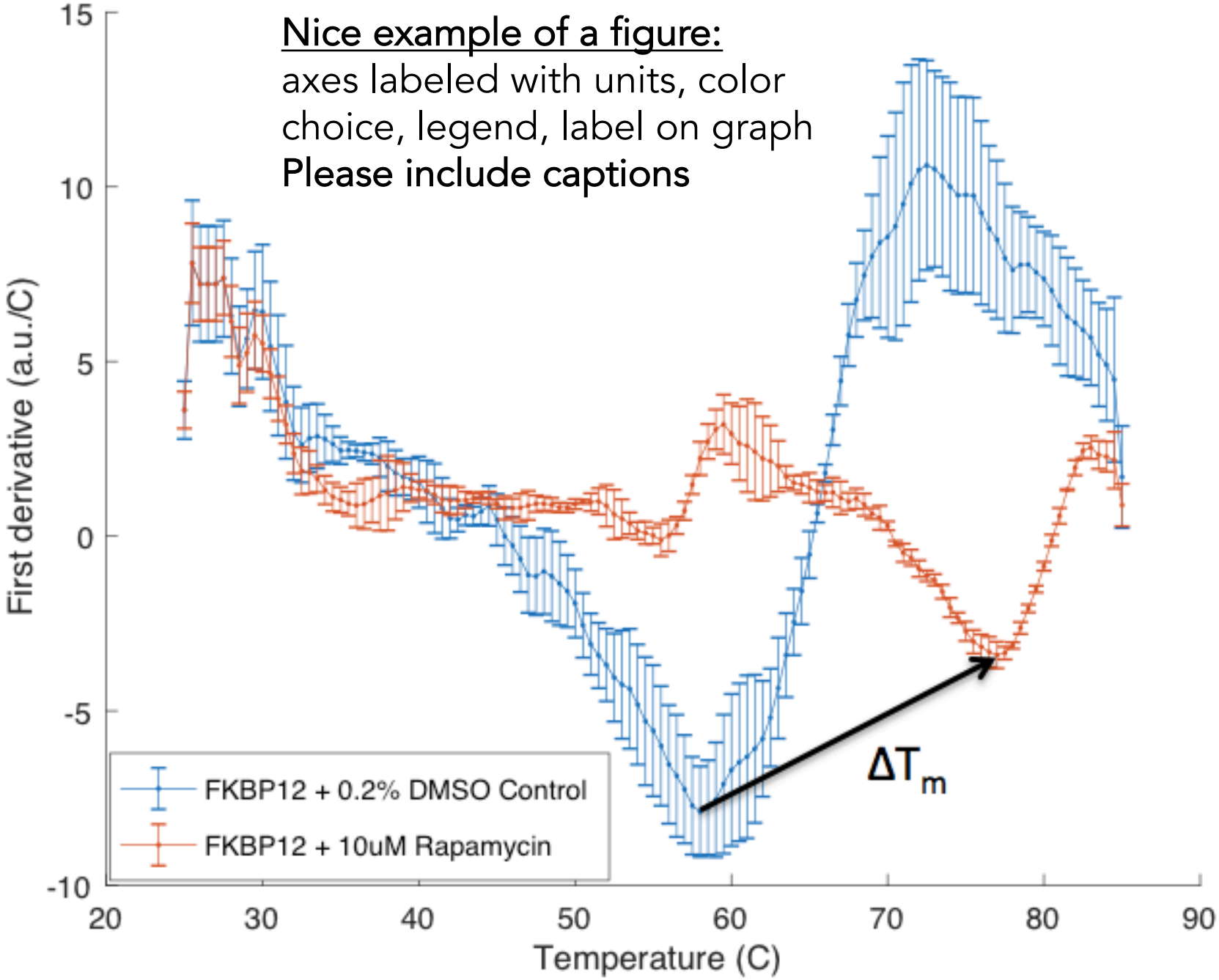
DSF thermal shift assay

Schematic: Experimental approach

Topic: Thermal shift/DSF assay design, samples tested

Figure: Raw thermal shifts or first derivative data plots for each condition tested (see Wiki for great example of Rapamycin vs. DMSO comparison)

Figure: Combined class data set for Rapamycin to determine an apparent affinity constant

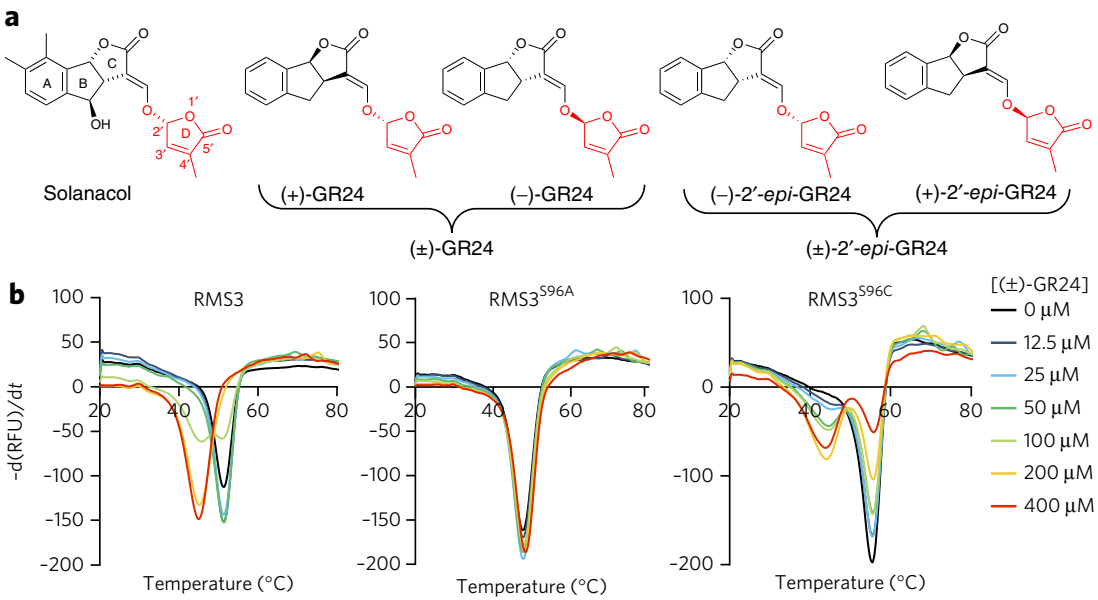

Nice example of a figure:
axes labeled with units, color choice, legend, label on graph
Please include captions
First derivative (a.u./C)
Temperature (C)
15
10
5
0
-5
-10
20
30
40
50
60
70
80
90
FKBP12 + 0.2% DMSO Control
FKBP12 + 10uM Rapamycin
ΔT m

ARTICLE
PUBLISHED ONLINE: 1 AUGUST 2016 | DOI: 10.1038/NCHEMBIO.2147

An histidine covalent receptor and butenolide complex mediates strigolactone perception

Alexandre de Saint Germain[1–6,14], Guillaume Clavé[7–9,14], Marie-Ange Badet-Denisot[7–9], Jean-Paul Pillot[1–4], David Cornu[10–12], Jean-Pierre Le Caer[7–9], Marco Burger[5,6], Frank Pelissier[7–9], Pascal Retailleau[7–9], Colin Turnbull[13], Sandrine Bonhomme[1–4], Joanne Chory[5,6]*, Catherine Rameau[1–4]* & François-Didier Boyer[1–4,7–9]*

Strigolactone plant hormones control plant architecture and are key players in both symbiotic and parasitic interactions. They contain an ABC tricyclic lactone connected to a butenolide group, the D ring. The DWARF14 (D14) strigolactone receptor belongs to the superfamily of α/β-hydrolases, and is known to hydrolyze the bond between the ABC lactone and the D ring. Here we characterized the binding and catalytic functions of RAMOSUS3 (RMS3), the pea (*Pisum sativum*) ortholog of rice (*Oryza sativa*) D14 strigolactone receptor. Using new profluorescent probes with strigolactone-like bioactivity, we found that RMS3 acts as a single-turnover enzyme that explains its apparent low enzymatic rate. We demonstrated the formation of a covalent RMS3-D-ring complex, essential for bioactivity, in which the D ring was attached to histidine 247 of the catalytic triad. These results reveal an undescribed mechanism of plant hormone reception in which the receptor performs an irreversible enzymatic reaction to generate its own ligand.



ARTICLE
PUBLISHED ONLINE: 1 AUGUST 2016 | DOI: 10.1038/NCHEMBIO.2147

An histidine covalent receptor and butenolide complex mediates strigolactone perception

Alexandre de Saint Germain[1–6,14], Guillaume Clavé[7–9,14], Marie-Ange Badet-Denisot[7–9], Jean-Paul Pillot[1–4], David Cornu[10–12], Jean-Pierre Le Caer[7–9], Marco Burger[5,6], Frank Pelissier[7–9], Pascal Retailleau[7–9], Colin Turnbull[13], Sandrine Bonhomme[1–4], Joanne Chory[5,6]*, Catherine Rameau[1–4]* & François-Didier Boyer[1–4,7–9]*

Strigolactone plant hormones control plant architecture and are key players in both symbiotic and parasitic interactions. They contain an ABC tricyclic lactone connected to a butenolide group, the D ring. The DWARF14 (D14) strigolactone receptor belongs to the superfamily of α/β-hydrolases, and is known to hydrolyze the bond between the ABC lactone and the D ring. Here we characterized the binding and catalytic functions of RAMOSUS3 (RMS3), the pea (*Pisum sativum*) ortholog of rice (*Oryza sativa*) D14 strigolactone receptor. Using new profluorescent probes with strigolactone-like bioactivity, we found that RMS3 acts as a single-turnover enzyme that explains its apparent low enzymatic rate. We demonstrated the formation of a covalent RMS3-D-ring complex, essential for bioactivity, in which the D ring was attached to histidine 247 of the catalytic triad. These results reveal an undescribed mechanism of plant hormone reception in which the receptor performs an irreversible enzymatic reaction to generate its own ligand.

summary table of data

Table 1 | Thermodynamic and kinetic constants of ligands and probes toward RMS3

Protein	RMS3							
Ligand	(±)-GR24	(±)-2′-*epi*-GR24	(+)-GR24	(−)-GR24	(±)-Solanacol	(±)-3′-Me-GR24	(±)-4′-Desmethyl-2′-*epi*-GR24	(±)-ABC
K_d (μM)	22.0 ± 4.8	71.0 ± 15.2	15.7 ± 3.7	35.9 ± 3.6	137.1 ± 33.2	30.9 ± 5.2	295.7 ± 27.9	271.2 ± 29.8
K_i (μM)	0.10 ± 0.07	0.23 ± 0.03	0.07 ± 0.01	5.17 ± 1.01	21.5 ± 2.6	n.d.	n.d.	28.8 ± 17.6
Protein	**RMS3**						**AtD14**	
Probe	**(±)-GC242**	**(−)-GC242**	**(+)-GC242**	**(±)-GC240**	**(±)-GC486**	**DiFMU**	**(±)-GC242**	
K_d (μM)	58.9 ± 9.6	82.6 ± 7.6	581.1 ± 194.3	74.1 ± 5.9	21.0 ± 1.4	19.9 ± 1.1	n.d.	
$K_{1/2}$ (μM)	0.49 ± 0.05	1.56 ± 0.32	17.42 ± 4.17	3.83 ± 1.80	n.d.	n.d.	1.19 ± 0.21	
k_{cat} (min^{-1})	0.012 ± 0.005	0.184 ± 0.017	0.136 ± 0.027	0.054 ± 0.015	n.d.	n.d.	0.030 ± 0.002	
$k_{cat}/K_{1/2}$ ($\mu M^{-1}\ min^{-1}$)	0.024	0.118	0.007	0.014	n.d.	n.d.	0.025	

Binding properties of RMS3 protein in the presence of different SL analogs were estimated by the apparent dissociation coefficients (K_d) derived from intrinsic fluorescence measurements. K_i values were determined using a competition test with (±)-GC242. $K_{1/2}$ and k_{cat} are pre-steady-state kinetic constants for RMS3 and AtD14 with different profluorescent probes. n.d., not determined. K_d values represent the mean ± s.e.m. of two replicates and $K_{1/2}$, k_{cat} and K_i values represent the mean ± s.e.m. of three replicates.

Implications and Future Work

Why is your work impactful and what would you do next?

Topic: Was your FKBP12 pure or active? If not, provide a putative explanation and explain how you might change the protocol if given another shot.

Topic: Did you have any compounds that confirmed as binders? Is this consistent with similar research? If not, provide a putative explanation.

Topic: How might you further validate that your SMM positive are binders and measure affinity values for the protein-ligand interaction? Other methods to complement DSF?

Topic: How can you use your FKBP12 binders to further research focused on this protein?

Topic: How might this method be improved?

Topic: How might this assay be used in the clinic? in industry?