Development of a Multiplexing platform for Molecular Glue Drug Discovery of Disease-Relevant High Value Undruggable Targets

Molecular-Glue Discovery & Development Team

EST. 2019

Heng Zhu, Ph.D.

Professor of Pharmacology and Molecular Sciences

Expertise: Targeted protein screening technology development 20+ years experience.

Seth S. Margolis, Ph.D.

Associate Professor of Biological Chemistry

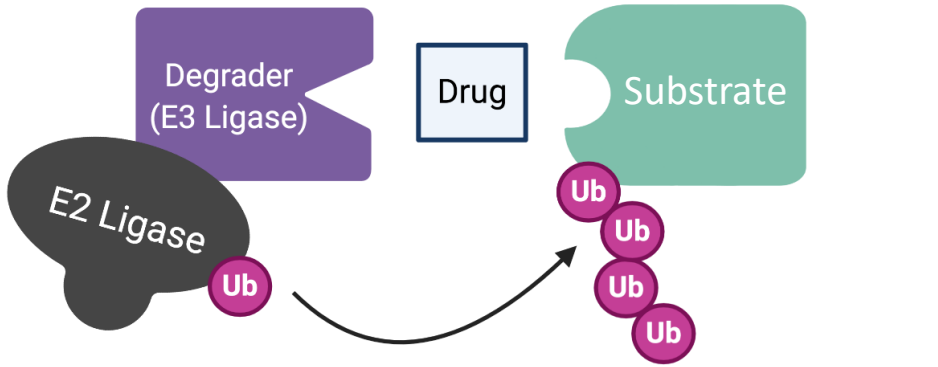
Associate Professor of Neuroscience

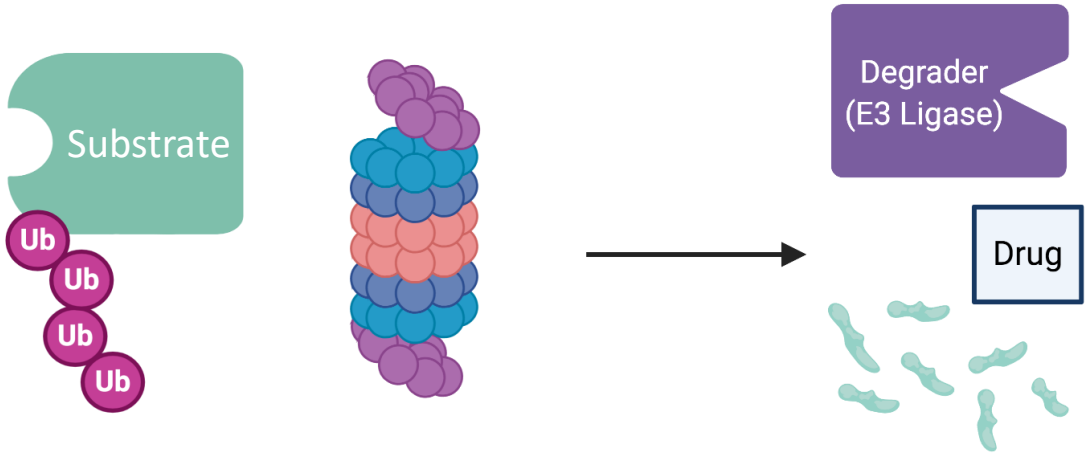
Expertise: Targeted protein degradation in the central and peripheral mammalian nervous system in health and disease 15+ years experience

Seeking Strategic Partner to Commercialize Technology

We seek a strategic partnership to help advance and commercialize the molecular glue technology and resulting therapeutics, through sponsored research and/or venture backed start-up

Targeted Protein Degradation: A new drug modality that uses the UPS.





Key points & advantages:

1) Hijack the normal cellular degradation machinery (UPS) to destroy a target protein in a specific manner.

2) Expands the druggable proteome to non-enzymatic proteins and those enzymes that have been deemed undruggable.

3) Potential inducible protein-protein interactions is very large….Requires high content multiplexing.

4) Surface area of protein allows for greatly increased drug target space.

High-throughput Molecular Glue Discovery & Development: Our Technology

Key points & advantages:

1) We have developed a high-throughput, multiplex assay to screen compound library against high-value disease targets that have previously been considered undruggable.

2) Increases rate of discovery of specific non-overlapping compounds.

3) Small scale reduces cost at the level of reagents and requirement for FTE.

4) A highly flexible system, allowing mix and match multiple targets vs. degraders.

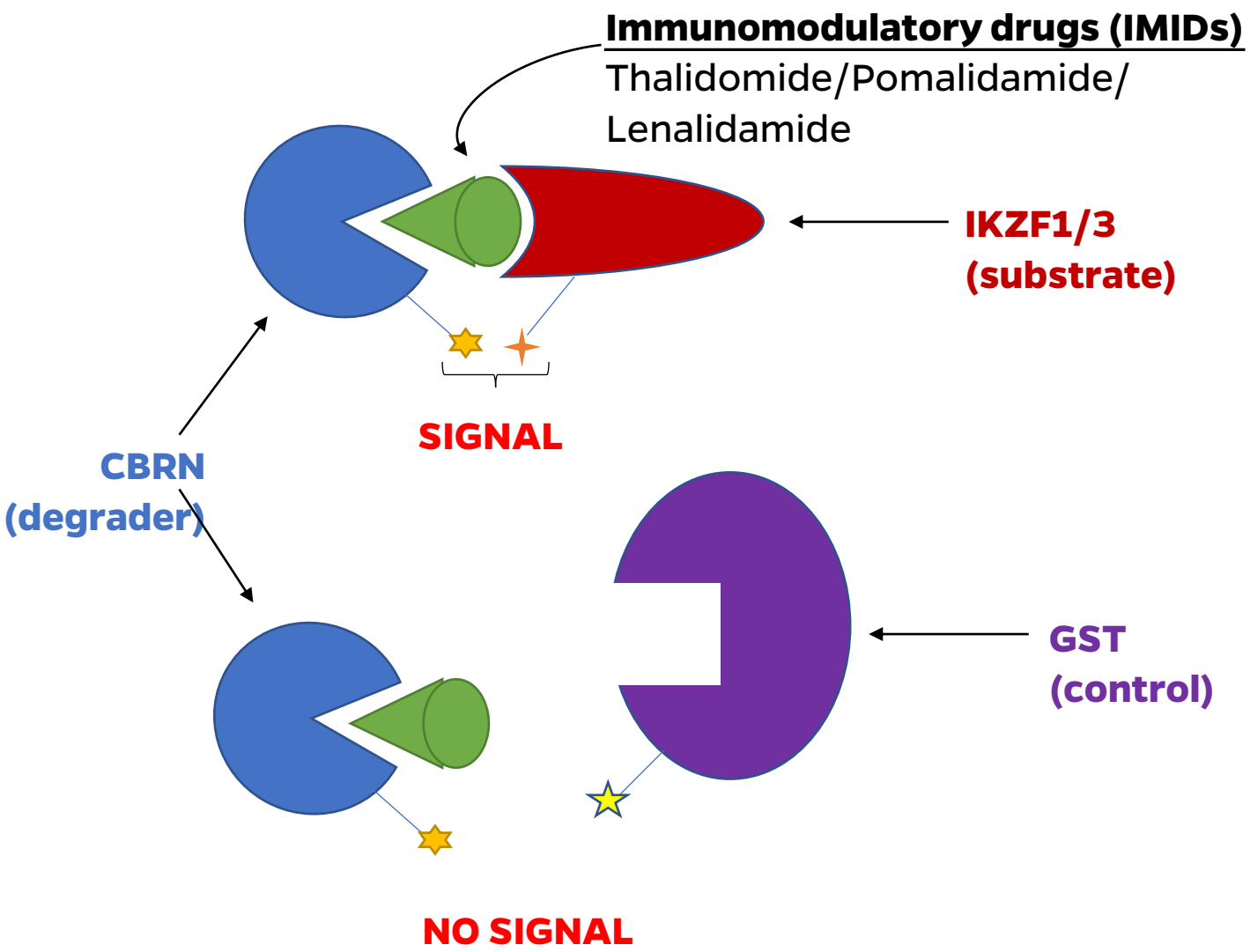
5) From set up to identification we anticipate a single week by one person and minimal reagents. Lead compounds ID < 3 months.

6) Lead compounds are refined, validated and screened in whole cell and then preclinical animal models.

7) *Adaptable to identify Molecular Glue degraders as well as Molecular Glue binders (another important capability for this tech)

8) *Easy to automate and scalable to hundreds of targets at once.

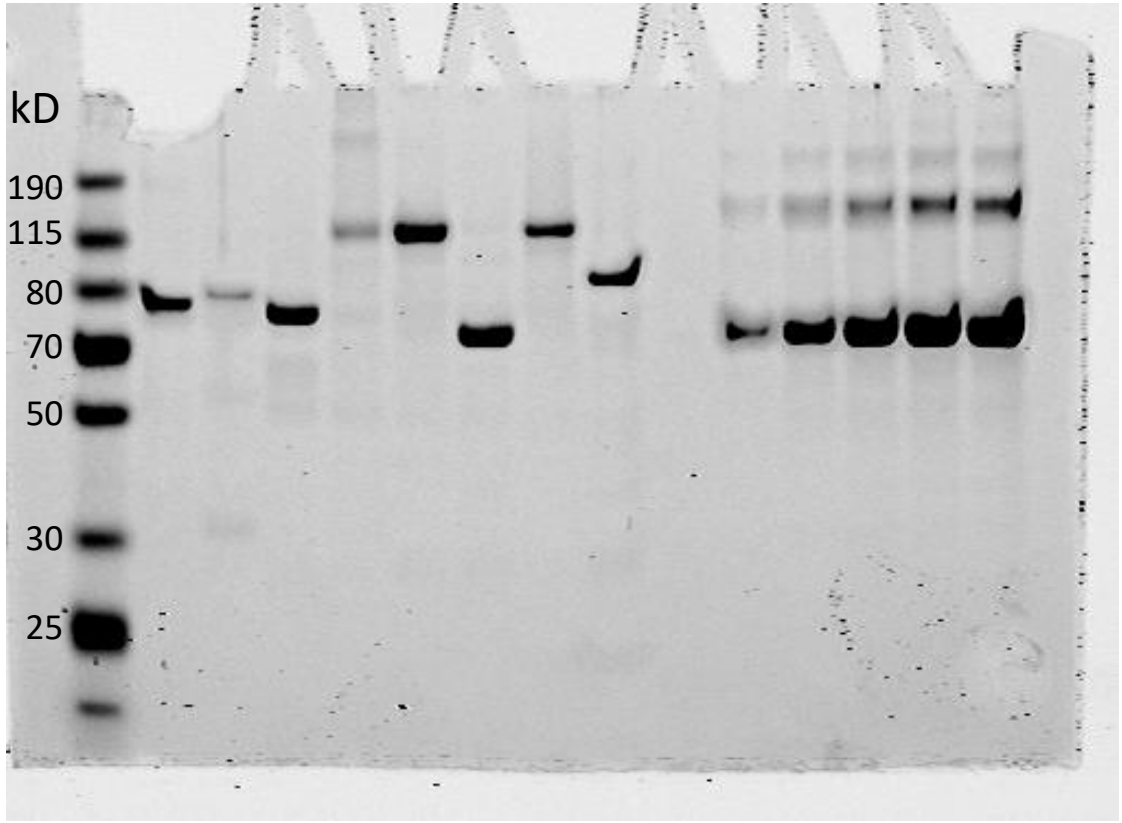
Technology Rationale



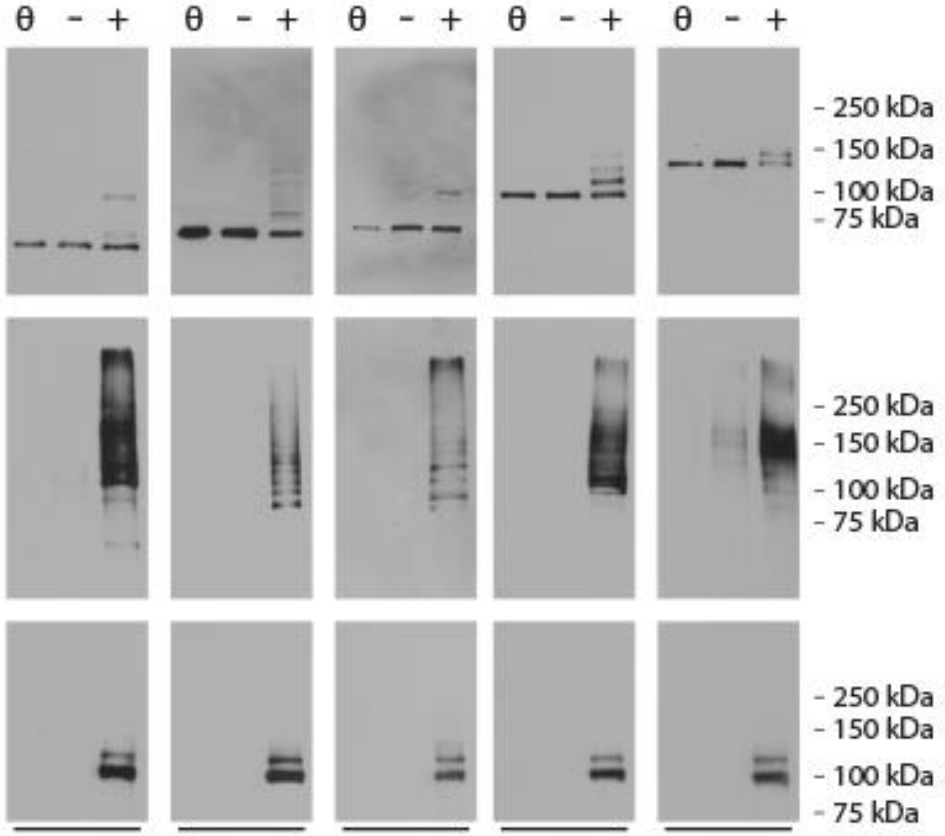
- IMIDs act as molecular glues forming a ternary complexes between the ubiquitin ligase Cereblon (CBRN) and the targets IKZF1/3
- The close proximity of the CRBN and IKZF1/3 in the presence of IMIDs produces a signal
- IMIDs do not form a ternary complex between CRBN and GST – no signal

Human proteome expression & purification: Making functional targets and degraders

Protein Purification



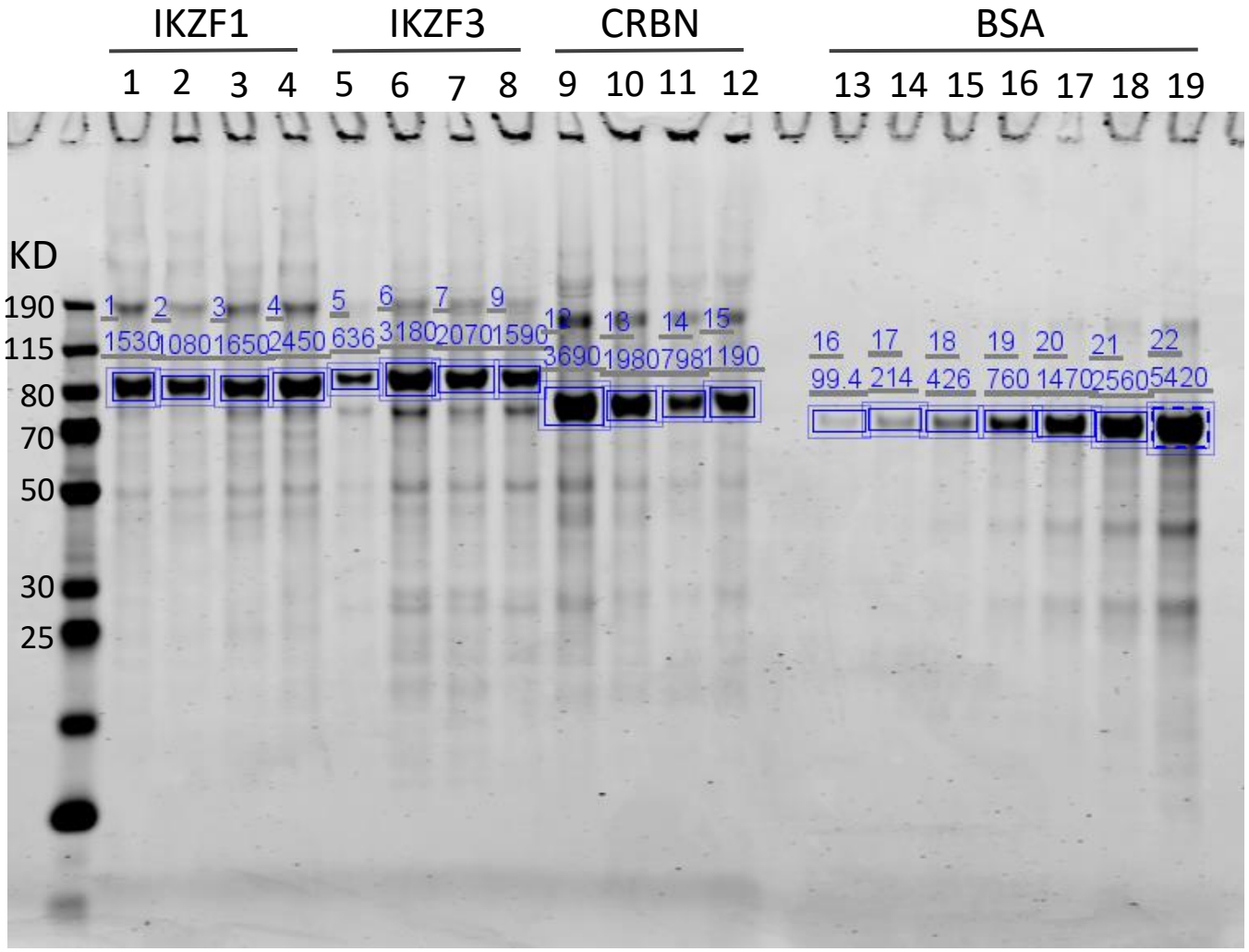
***In vitro* ubiquitination assays**



JHU ASSET: > 20,000 human proteins to choose from

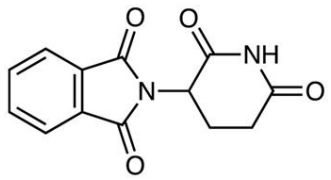
JHU CAPABILITIES: Purifying limitless quantities of human E3's and target proteins

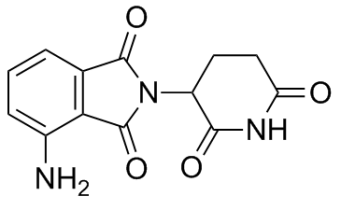
PHASE A.1 – Purified known target proteins

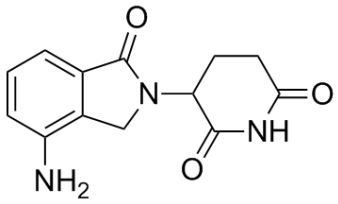


	Protein	M.W.	Yield (µg)	pmol
1	IKZF1	78.71	24.95	316.97
2	IKZF1	78.71	17.61	223.74
3	IKZF1	78.71	26.90	341.83
4	IKZF1	78.71	39.95	507.56
5	IKZF3	84.02	10.37	123.42
6	IKZF3	84.02	51.85	617.10
7	IKZF3	84.02	33.75	401.70
8	IKZF3	84.02	25.92	308.55
9	CRBN	76.55	60.17	786.02
10	CRBN	76.55	32.28	421.77
11	CRBN	76.55	13.01	169.98
12	CRBN	76.55	19.40	253.49
13	BSA (0.03125ug)	66.46	0.031	0.470
14	BSA (0.0625ug)	66.46	0.063	0.94
15	BSA (0.125ug)	66.46	0.125	1.89
16	BSA (0.25ug)	66.46	0.25	3.76
17	BSA (0.5ug)	66.46	0.5	7.523
18	BSA (1ug)	66.46	1.0	15.05
19	BSA (2ug)	66.46	2.0	30.10

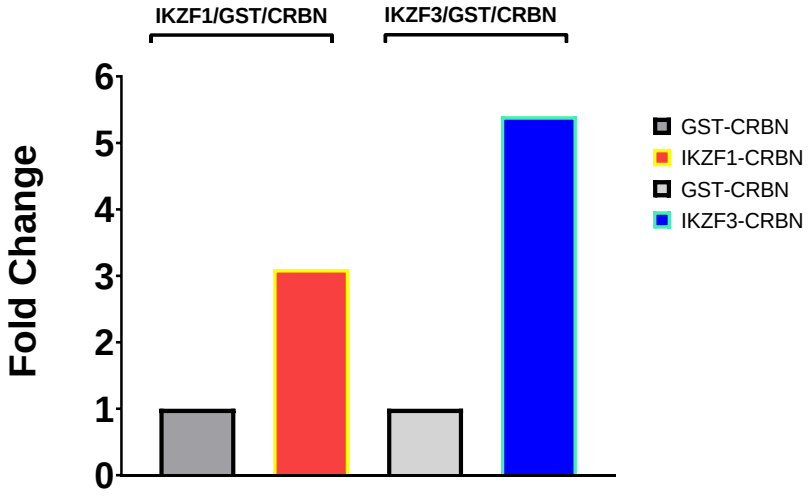
PHASE A.2 – Assessed known CRBN/IMID interactions



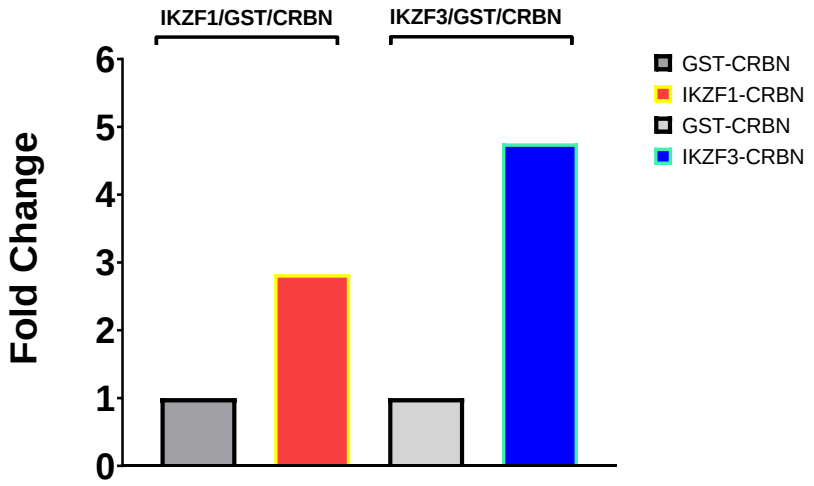




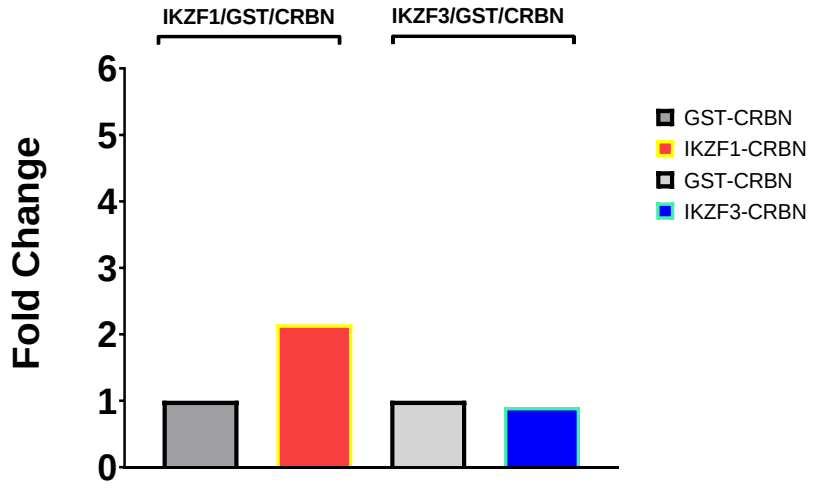
Thalidomide



Pomalidomide



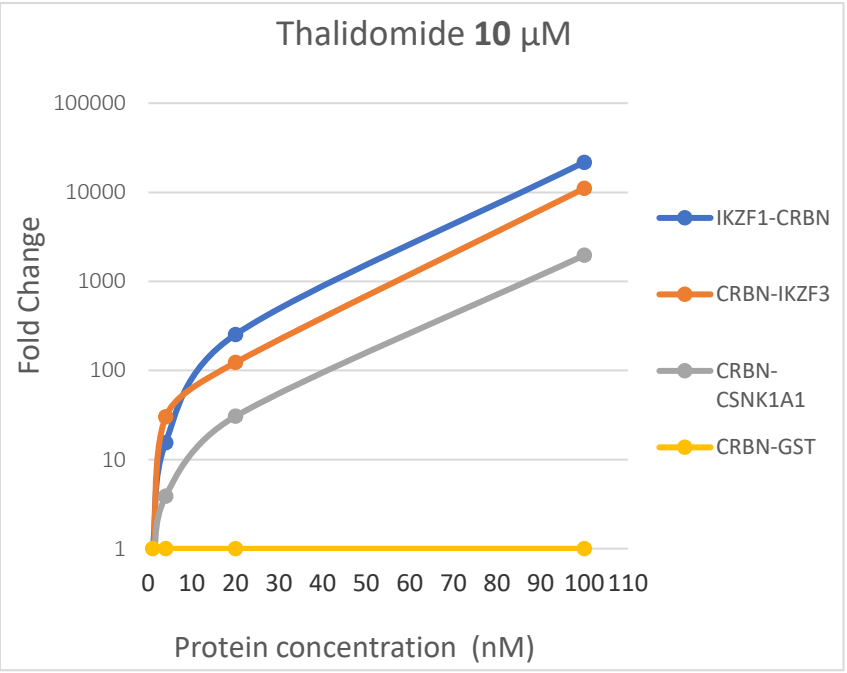
Lenalidomide

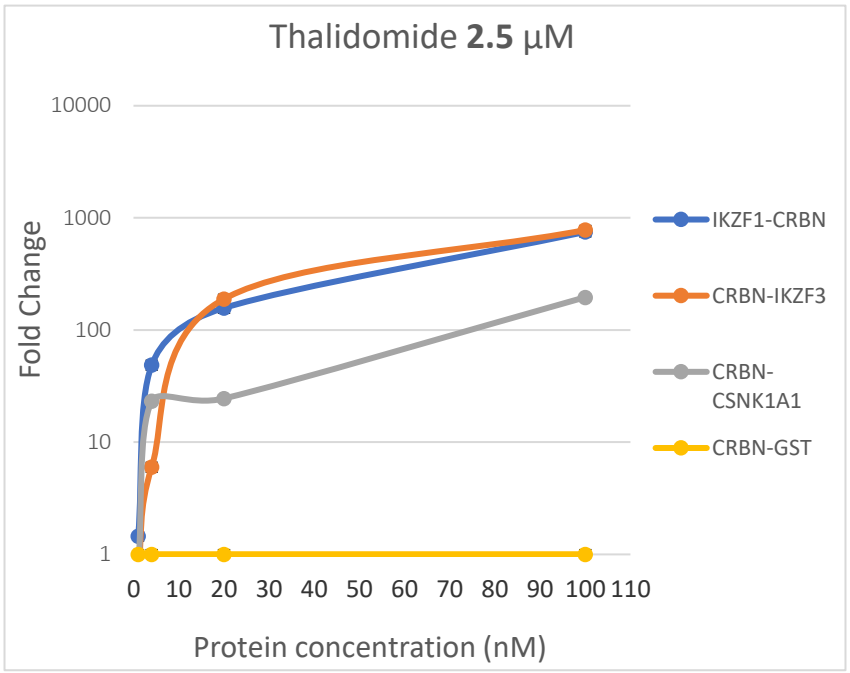


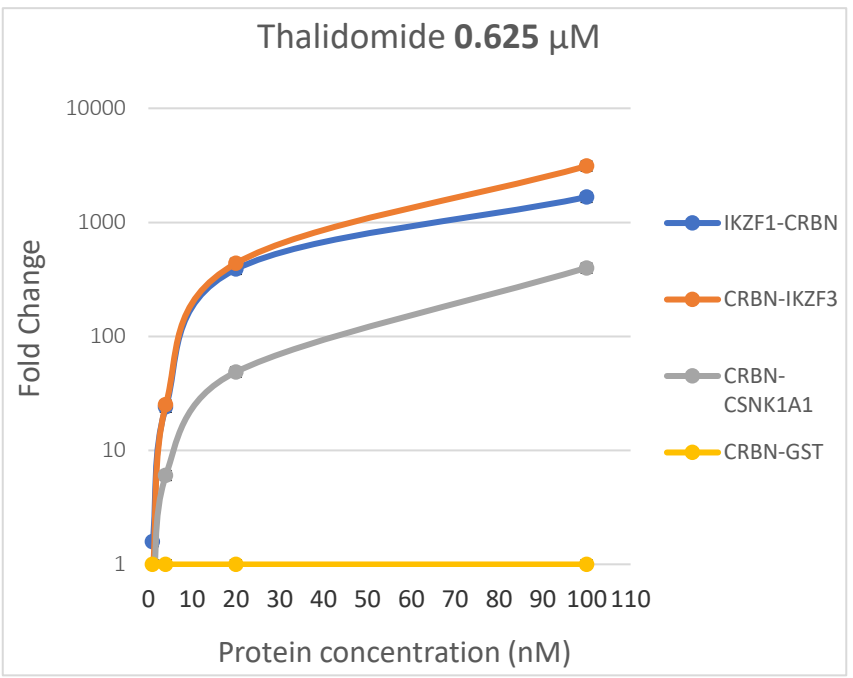
JHU CAPABILITIES: MG discovery assay with several key features:

- Single well - all-in-all multiplexing
- High resolution and specific substrate discrimination
- High resolution and selective discrimination of IMID Interactions

PHASE A.3 – Optimized multiplexing: CRBN mixed with IKZF1, IKZF3, CSNK1A1 & GST



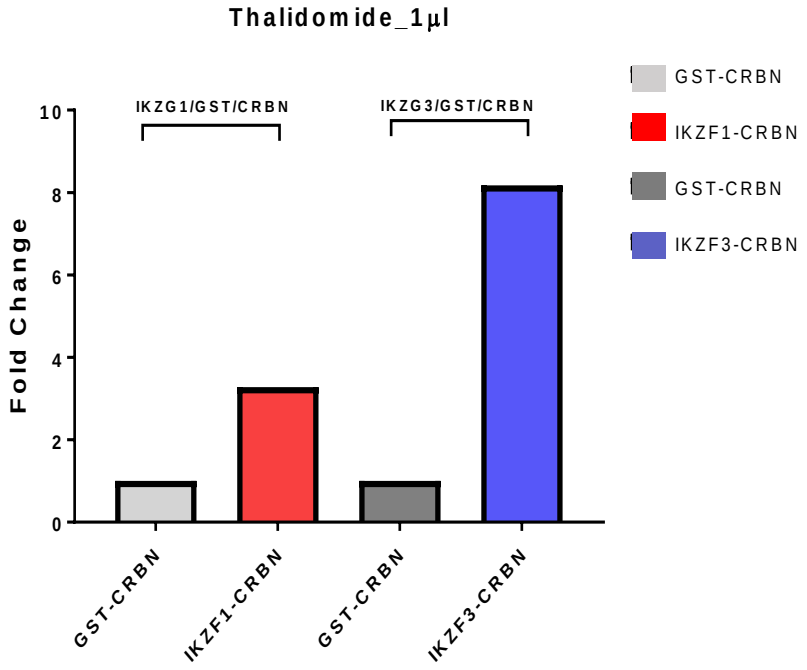


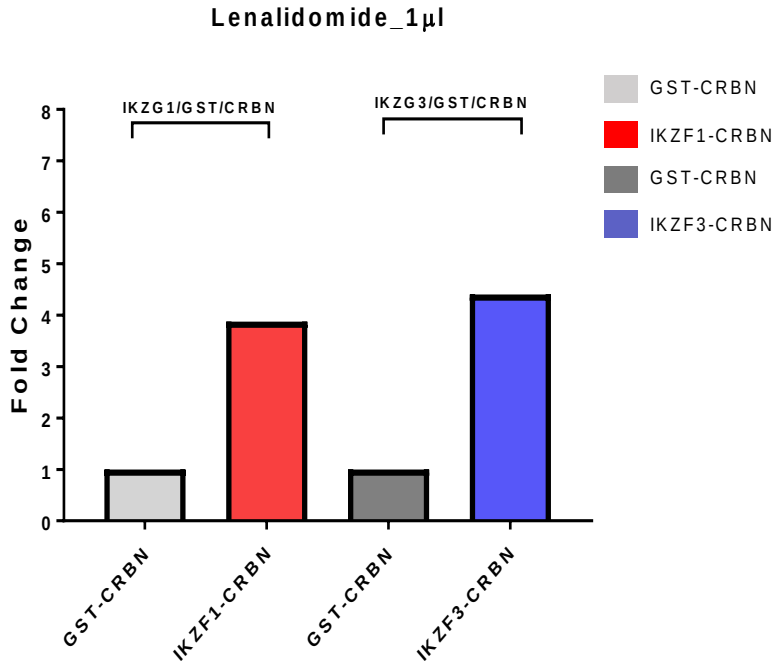


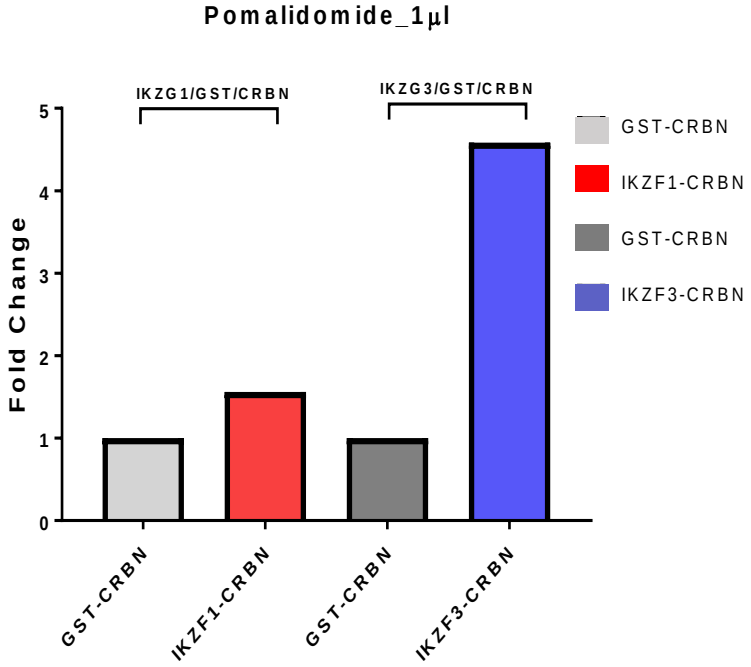
JHU CAPABILITIES: Key features determined in optimization:

- Target preference (IKZF1/3 › CSNK1A) detected, an advantage of all-in-all format
- Cost-effective – Protein concentrations pushed down to low nM
- Ternary complex not likely affected by MG concentration, avoiding hook effect

PHASE A.4 – Reduced to 1ml reaction volume using Echo 650







JHU CAPABILITIES: Key features in automation:

- Low reaction volume and sample consumption
- Amenable to 384-well format
- High signal-to-noise ratio maintained
- Feasible for HT drug screening

PHASE B – HT novel MG discovery & optimization

JHU ASSET
Curated a list of high priority and disease relevant E3 ligase/substrate pairs

JHU ASSET
Large scale pooled small molecule library

JHU ASSET
Proprietary deconvolution strategy

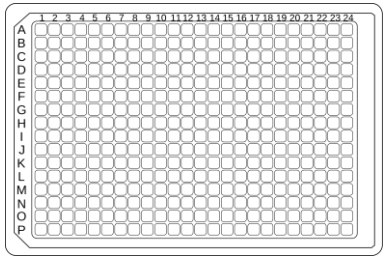
JHU ASSET
MG discovery and optimization of assay

PHASE B.1 – Purified E3 ligases and disease target proteins (Cancer & Neurodegeneration)

PHASE B.2 – HT MG screening pipeline

Target & ligase Mixture

Echo 650



384-well plate

Deliver mini pools of drugs to well

Incubate

Transfer

Screen for E3/drug/target ternary complexes

Data collection

& analysis

Identify specific MGs

PHASE C – Analyzed data to identify compounds that mediate specific ternary complexes (~3 months from start to Phase C)

Plate 1

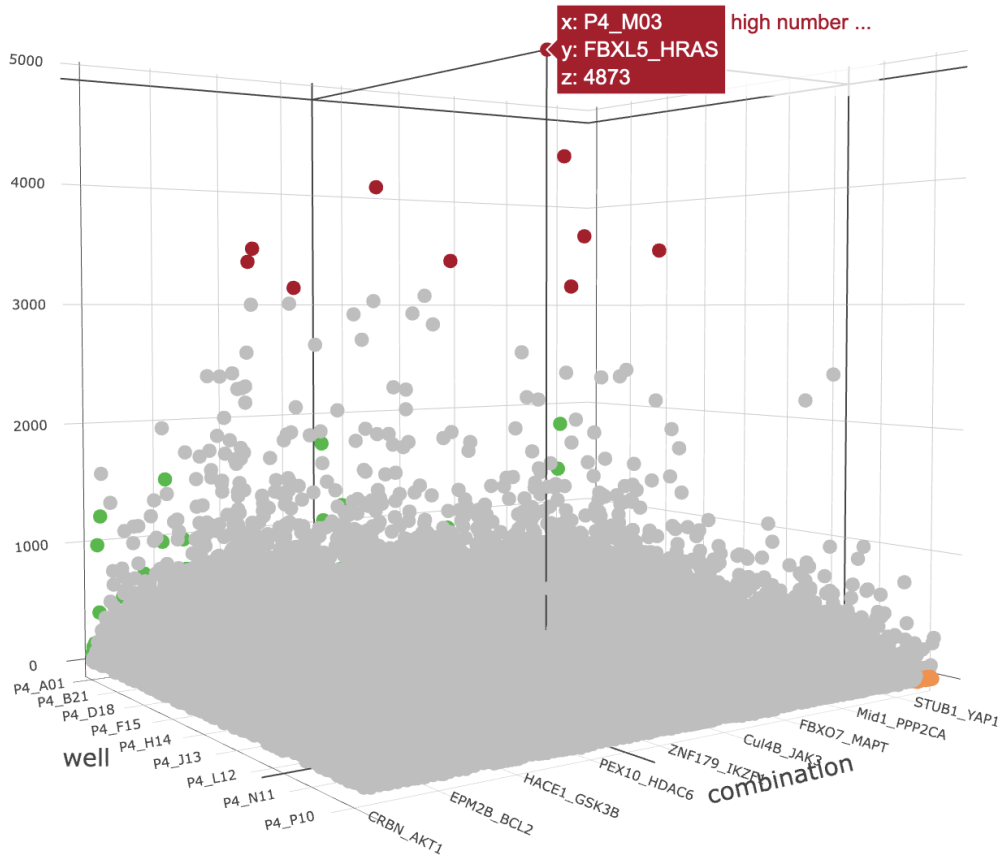
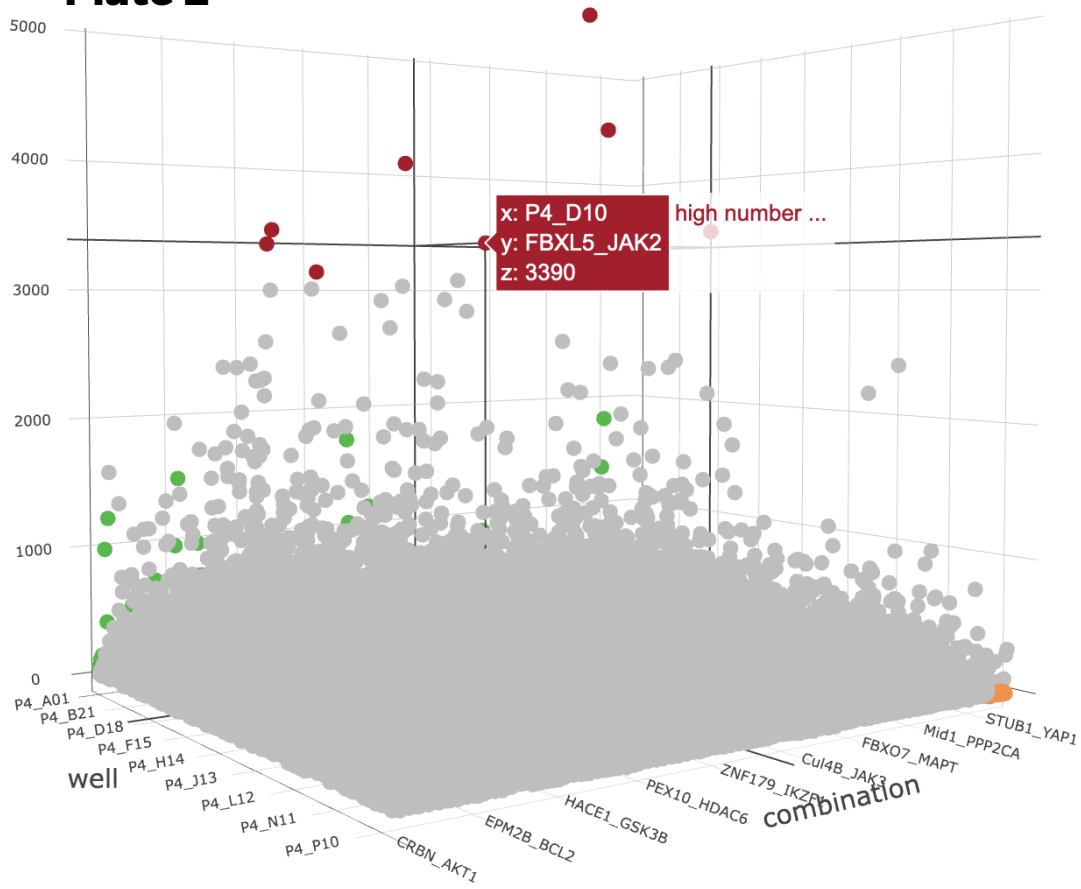


Plate 2



In each well we examined 378 (=14 ligases X 27 target proteins) combinations against a mini pool of 8 compounds. Therefore, we surveyed 472,500 events in total (=378 X (10k/8)).

SUMMARY

- **Phase A:** Accomplished the POC of detecting MG-dependent ternary complex formation
- **Phase B:** Developed and optimized a highly multiplexed, HT screening pipeline for searching MGs
- **Phase C:** Identified novel MGs for specific E3-target pairs
- **Phase D:** Validate compounds

Next Steps

Establish strategic partnership with investors and/or corporate partners

In the pipe-line

- Using our proprietary approaches we will prioritize each well with a unique ternary complex formation
- Deconvolute wells to identify specific compound
- Perform high content degrader screen for each hit compound and ligase/substrate pair.

Medium-term goals

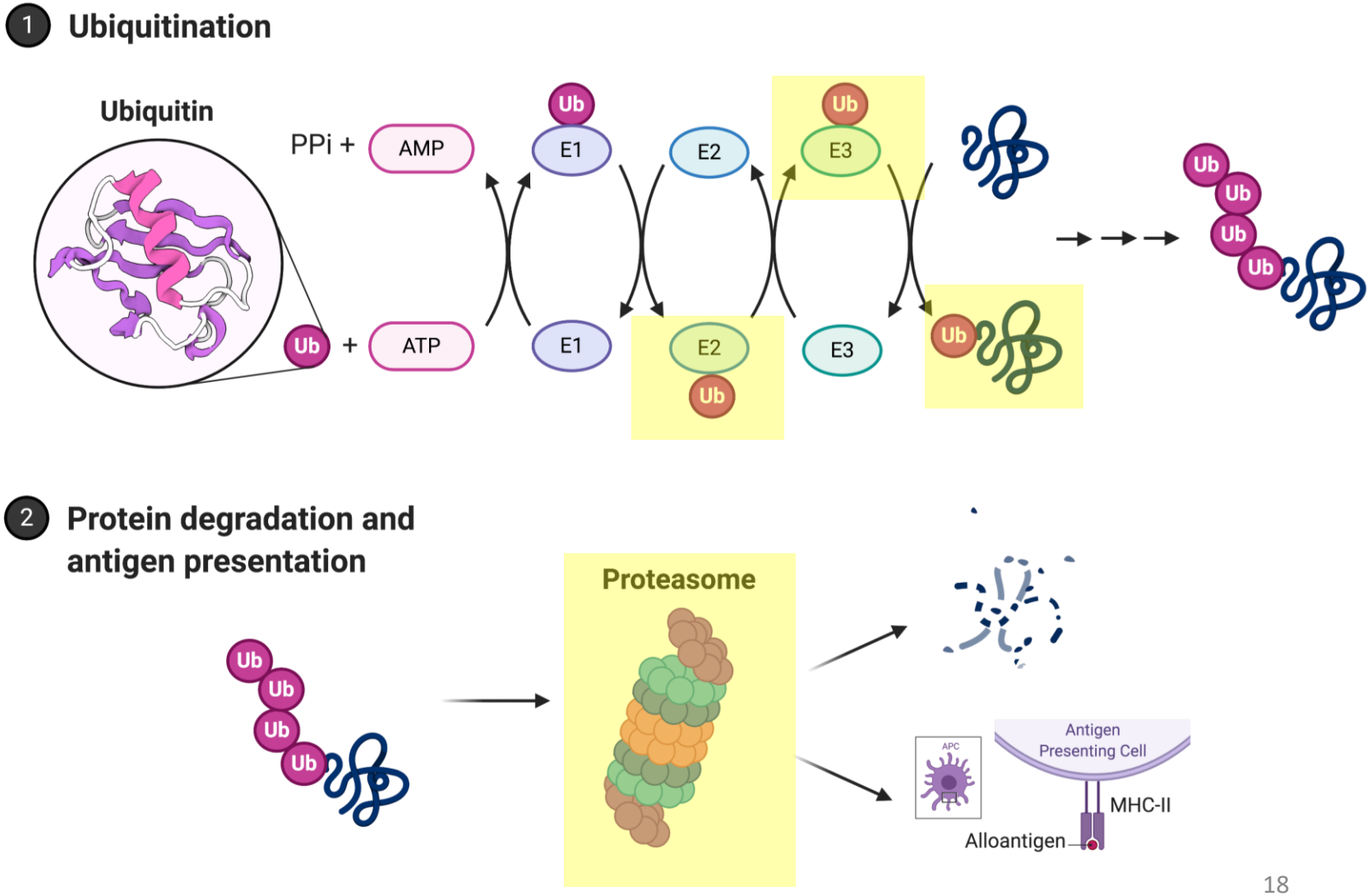
- Identify new lead compounds for strategic partners interested in specific targets (**can start immediately**)
- Establish New-co to bring new degraders to clinical use (**investment needed**)
  - High value compounds will be further tested in respective disease models.
  - Quantitative proteomics will be used to assess target destruction in vivo.
  - Possible need for SAR to proceed with hits.

Appendix

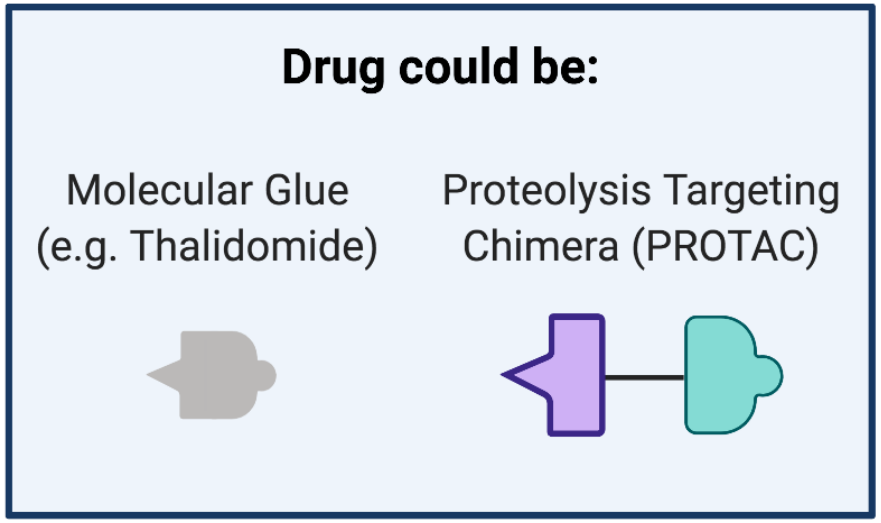
The Ubiquitin Proteasome System (UPS) degrades proteins in all mammalian cells.

2 Major Steps:

Key Players highlighted yellow:



Targeted Protein Degradation: PROTAC vs Molecular Glue



PROTAC are the current hot market.

- They are bi-functional Molecular-Glues.
- They are large and bulky.
- They take many steps to develop.
- Long lead time to realization of specificity and effectiveness.
- Expensive

Molecular Glue.

- Single bi-functional compound.
- They are small with better PK/PD.
- Better cell penetration.
- They require assays to to detect the ternary complex (Target-Glue-Degrader E3)
- No one has developed such an assay until **Now**!

The molecular glue space is very large…High content multiplex platforms are essential

Protein – protein interactions

- Number of proteins: ~100,000
- Known protein interactions: ~130,000 and 600,000
- Possible interactions: 5 X 10^9

Targeting surface area of proteins

- Radius of average protein: ~2.6 nm
- Surface area average protein: ~85 nm^2
- Average size of small molecule: 1 nm