

Molecular Glues: New Frontier for Drug Discovery

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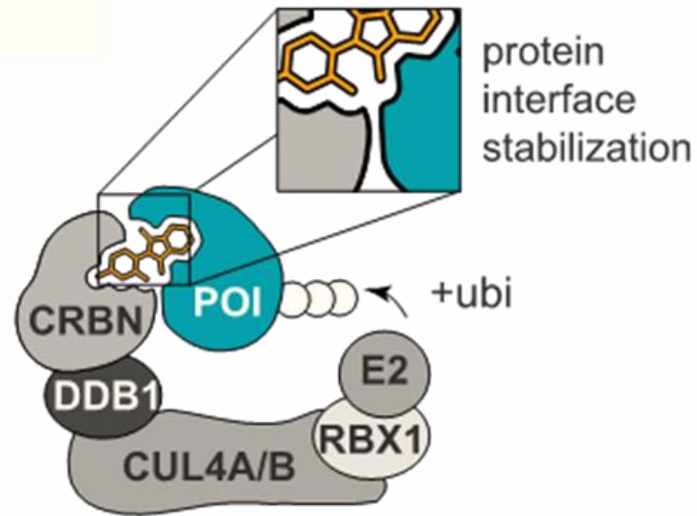
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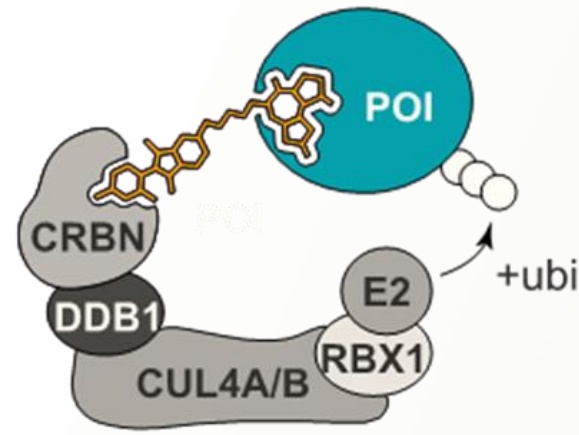
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Molecular Glue vs PROTACs



Molecular Glue

**New Protein-protein interactions in the cell.
Ubiquitination of neo-substrates**



PROTAC

**Expectations for PROTACs are very high, but
there are developmental challenges**

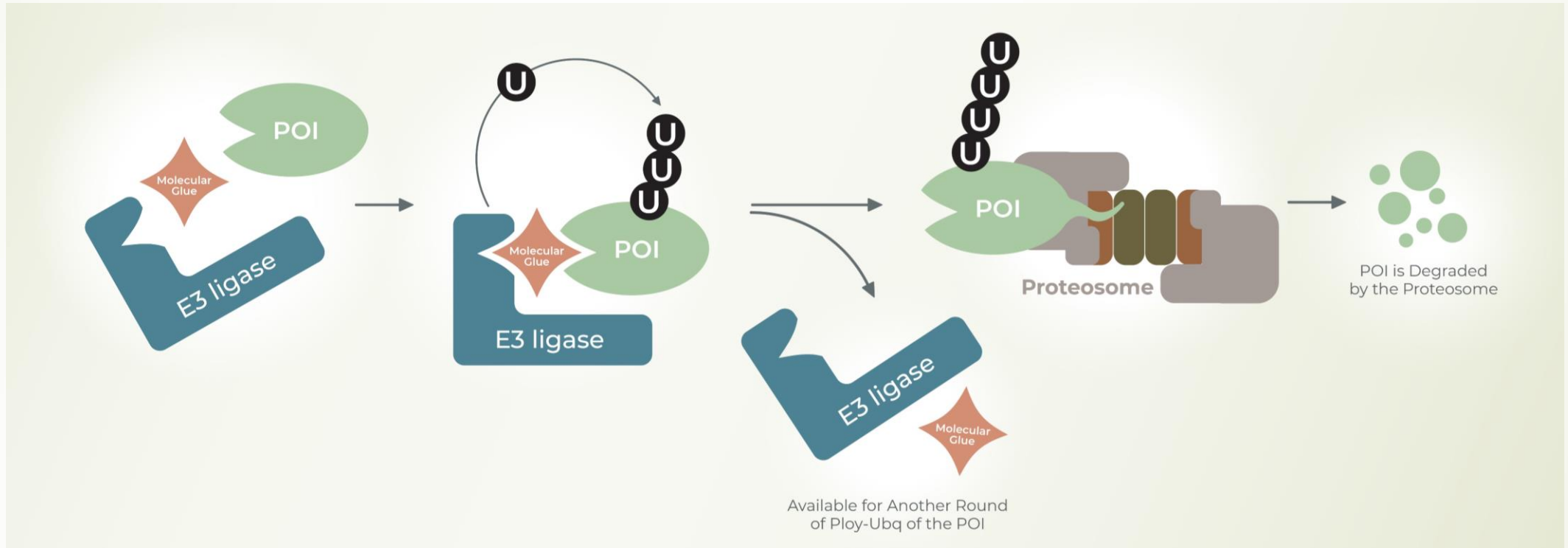
Image credit: <https://www.protein-degradation.org/groups/winter/>

Molecular Glues, Vast Applications

Small molecules that selectively change confirmation of proteins to:

- Bind to ubiquitin E3 ligases and promote ubiquitination of neo-substrates
- Bind to target proteins, in proximity of ubiquitin ligases, promote ubiquitination of target proteins
- Depending on the Glue, E3 ligase may promote compartmentalization, loss of function or stabilization of target proteins

Degrader Molecular Glues

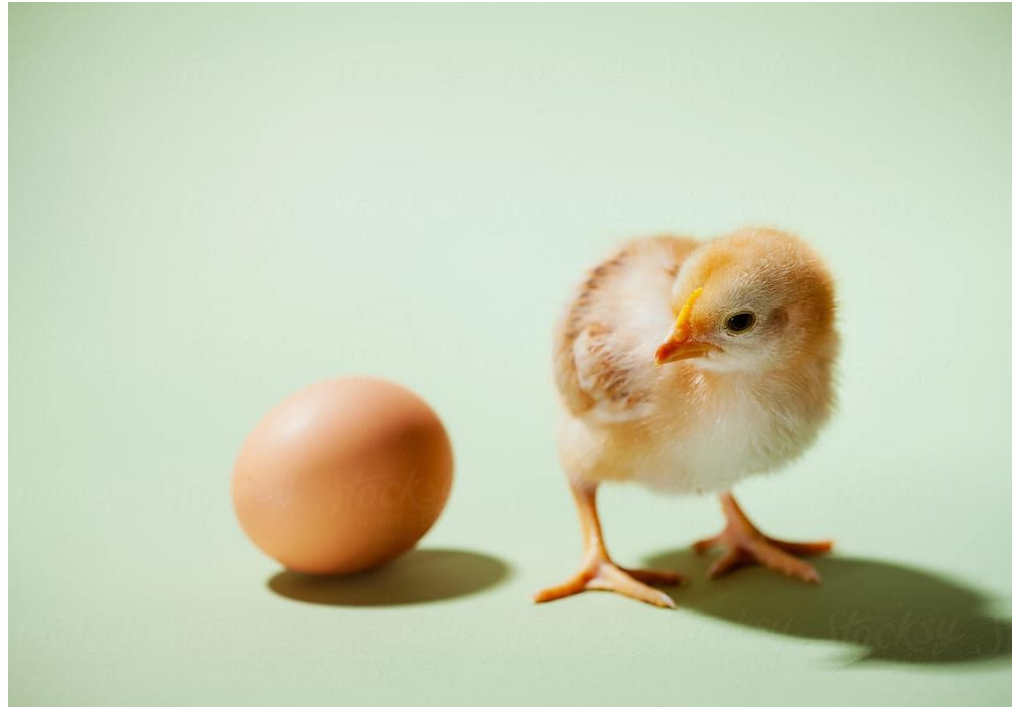


**It Took 60 Years, Tragedies and Serendipities to Discover
Thalidomide Molecular Glues**

Current Approaches for Discovering Molecular Glues

- **Proximity Assays: Empirical screening of E3 ligases to degrade target proteins**
- **Application of LifeSensors [TUBE technologies](#) to directly discover/analyze ubiquitination in vitro**
- **LifeSensors microtiter plate-based HTS to monitor mol glue mediated ubiquitination in cells**
- **Cell-based and reporter-based screens (prone to artifacts as reporters contain lysines)**
- **Structural biology and AI based screens**

Rational Way to Screen for Molecular Glues

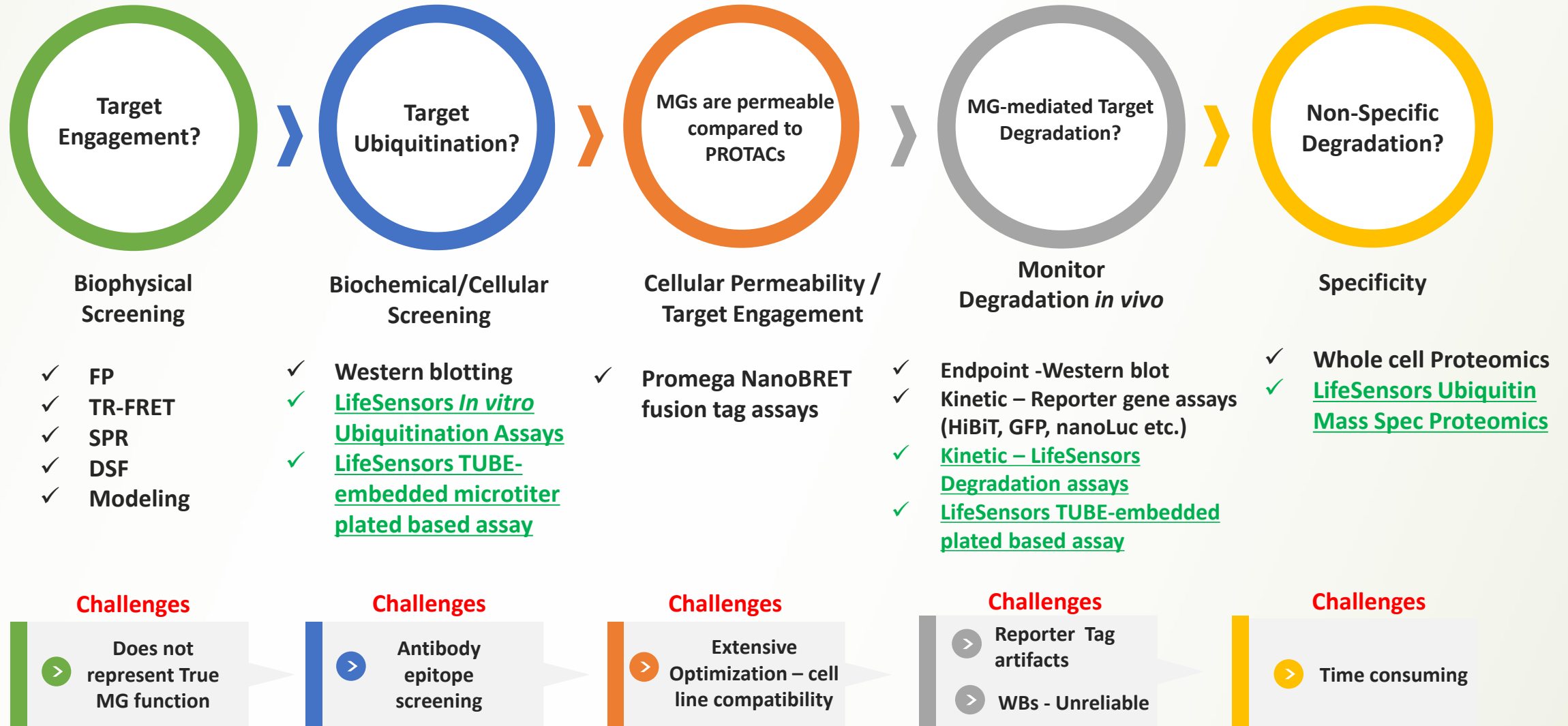


Monitor Ubiquitylation First

Differentiating Molecular Glues and Traditional PROTACs

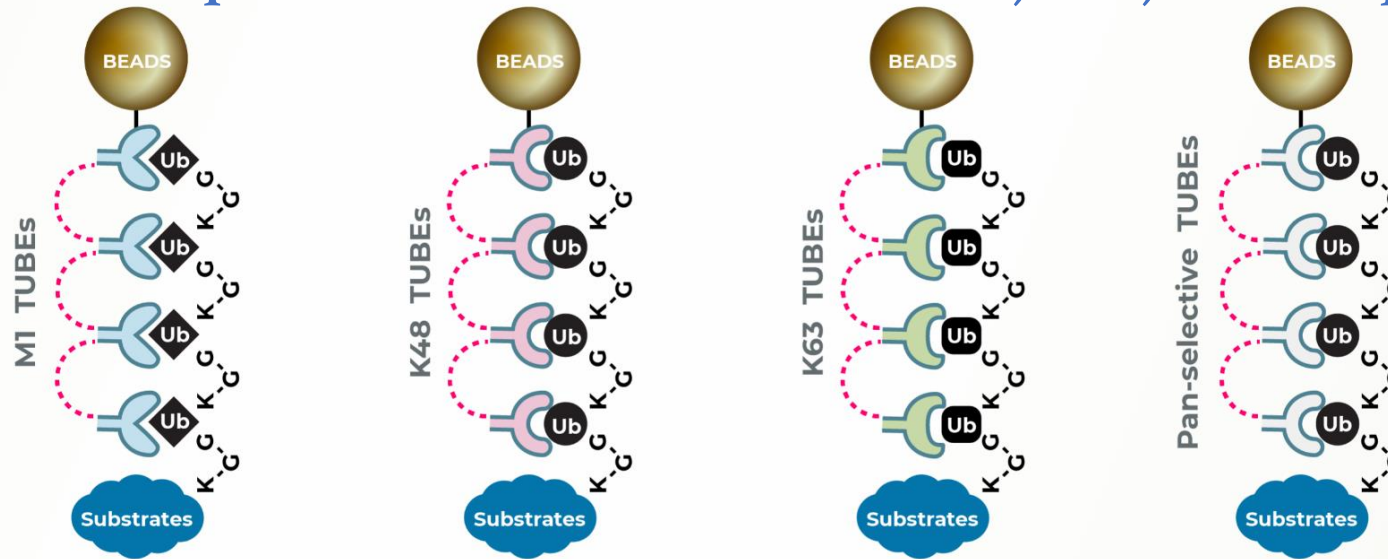
MOLECULAR GLUES	VS	PROTACS
Monovalent	FEATURE	Bivalent
No	LINKER	Yes
<500 Da	MOLECULAR WEIGHT	700-1300 Da
Within	LIPINSKI'S RULE OF 5	Defy
To be screened	TARGET	Predictable
Not required	BINDING POCKET	Required
Weak binders function well	BINDING AFFINITY	Strong binders function well

Current Tools for Molecular Glue Research

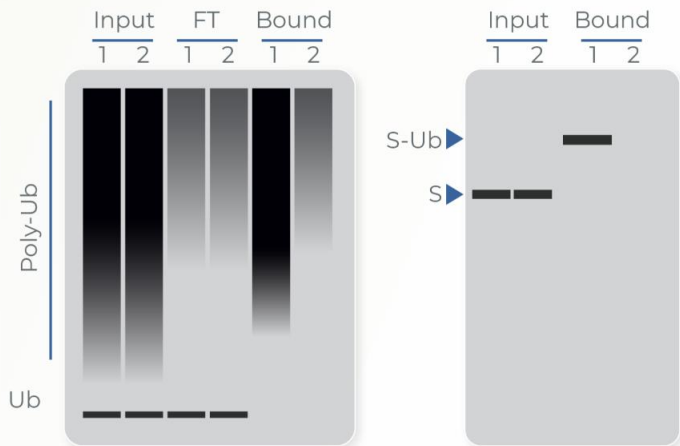


TANDEM UBIQUITIN BINDING ENTITIES (TUBEs): Applications in UPS

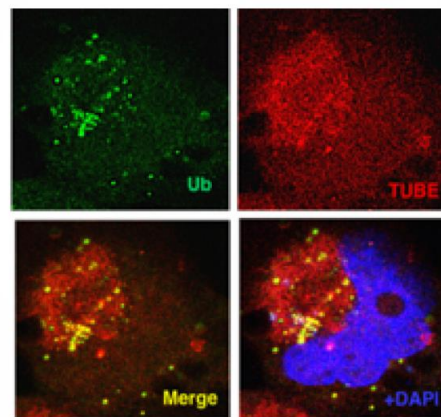
Imaging & Isolation of Ubiquitinated Proteins to Monitor Pan, K48, K63 Ubiquitination, *In vitro*, *In vivo*



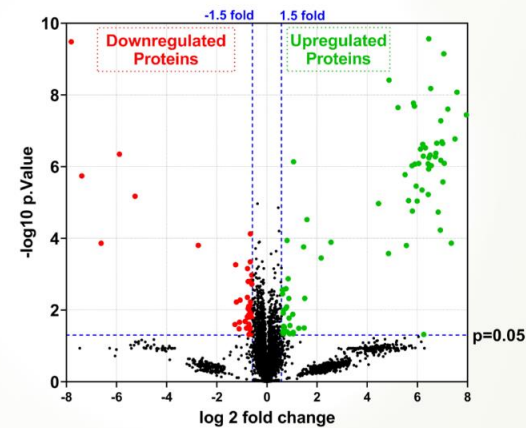
Poly-Ubiquitin Substrate Enrichment & Western Blotting



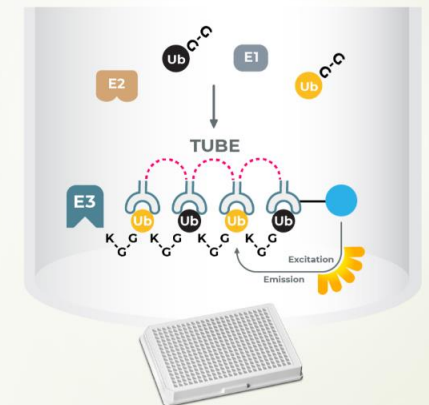
Fluorescent Microscopy



Mass Spectrometry Ubiquitomics

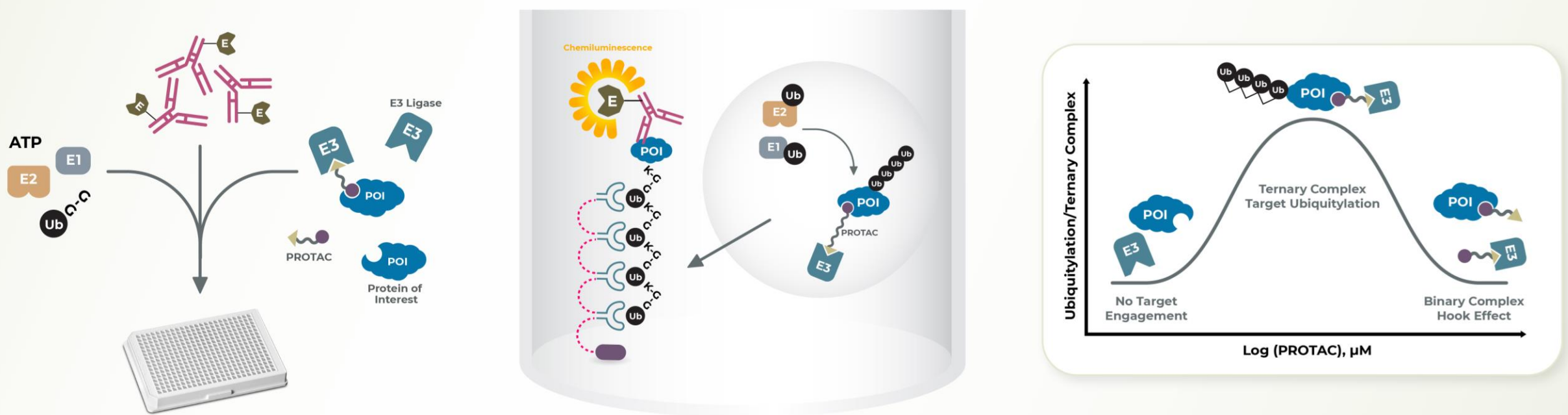


High-Throughput Screening



HTS- In Vitro MGs/PROTAC Screening Platform

Monitor Molecular Glue Mediated Ubiquitination



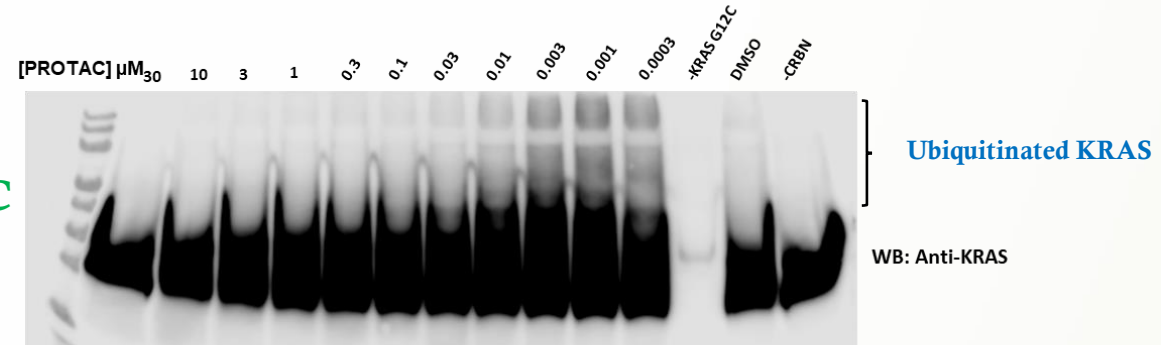
TUBE Capture & PROTAC Mediated Ubiquitination of POI Detection

CRBN & VHL KRAS Degraders

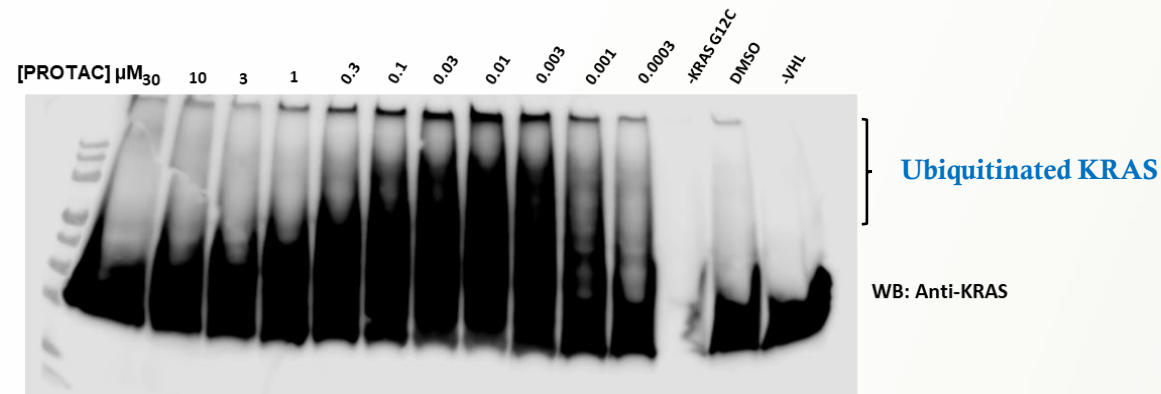
Monitor Molecular Glue Mediated Ubiquitination of KRAS G12C vs G12D in vitro, – Western blotting

Please note ligase and PROTAC mediated ubiquitination increases the molecular weight of K-RAS on gels

**CRBN KRAS G12C
Degradar**

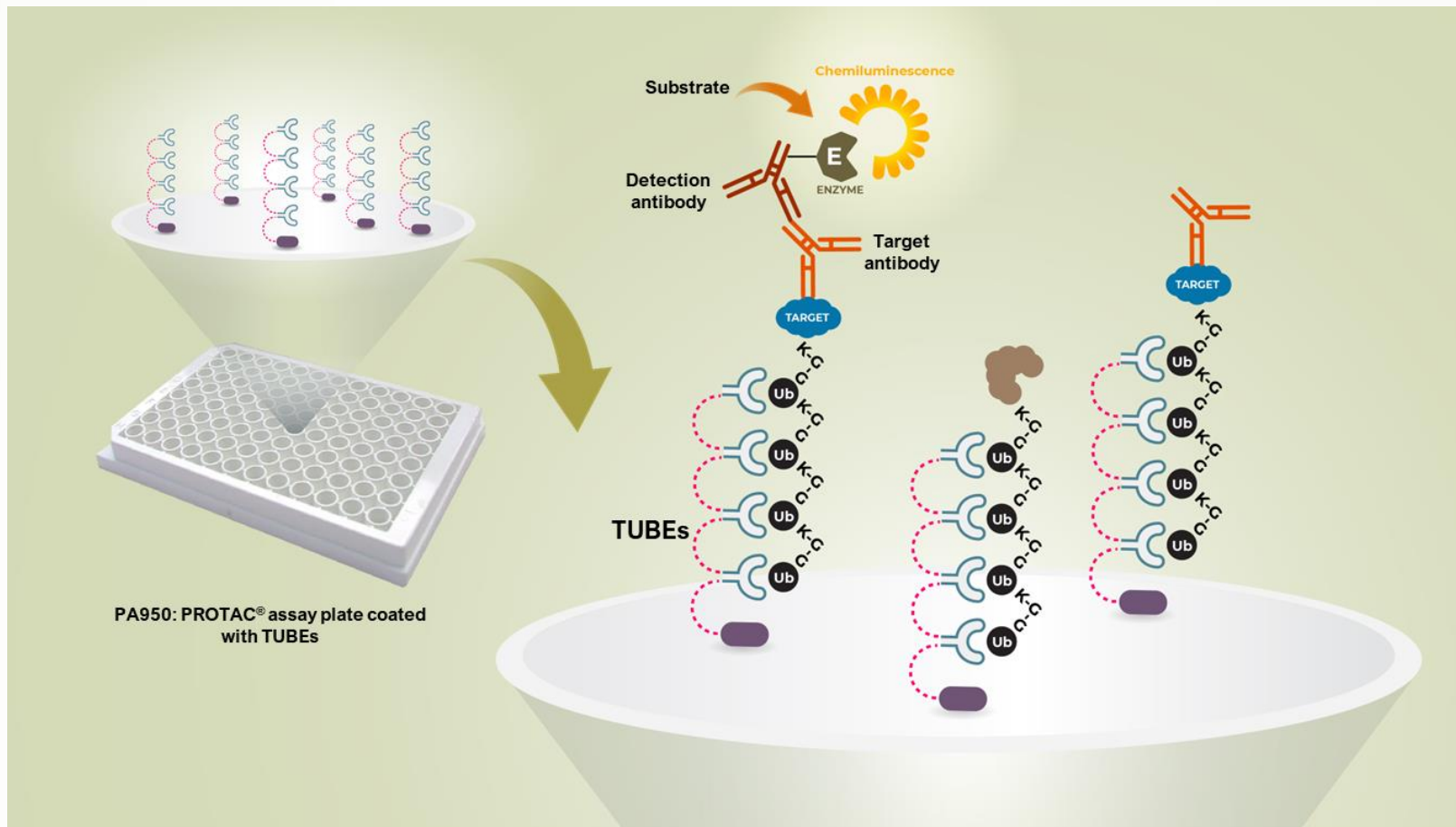


**VHL KRAS G12C
Degradar (LC2)**



Ubiquitination Assays– Cell Based Assays

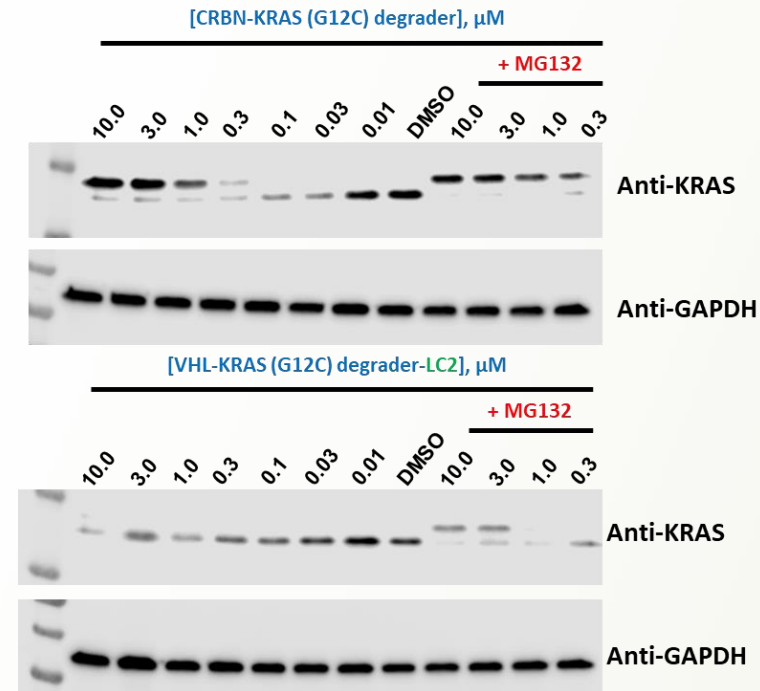
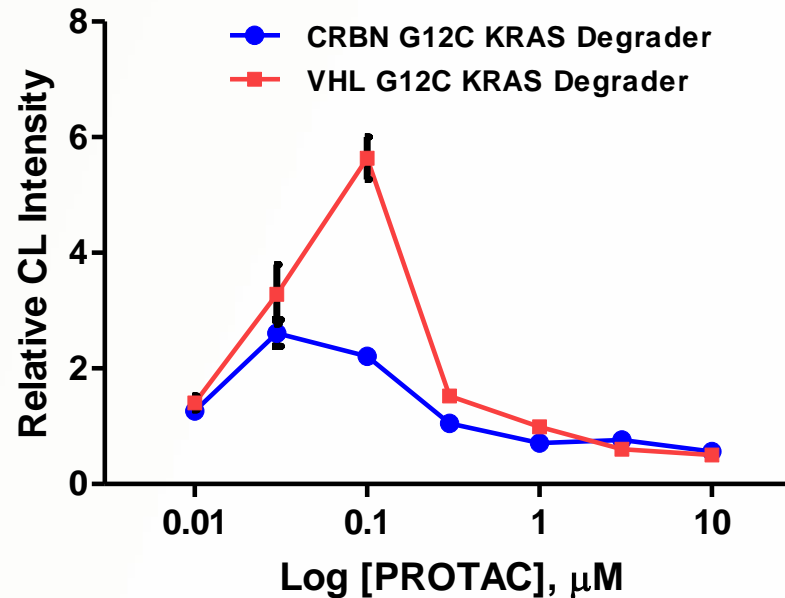
Monitor Molecular Glue Mediated Ubiquitination and Degradation by HTS on TUBE Microtiter plates



CRBN & VHL KRAS Degraders

Monitor PROTAC mediated Cellular Ubiquitylation and Degradation Microtiter Plate base or Western Blotting

Dose Response Study - HTS

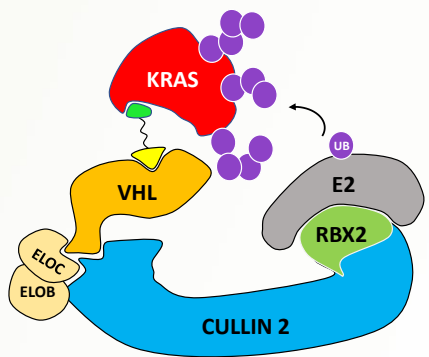
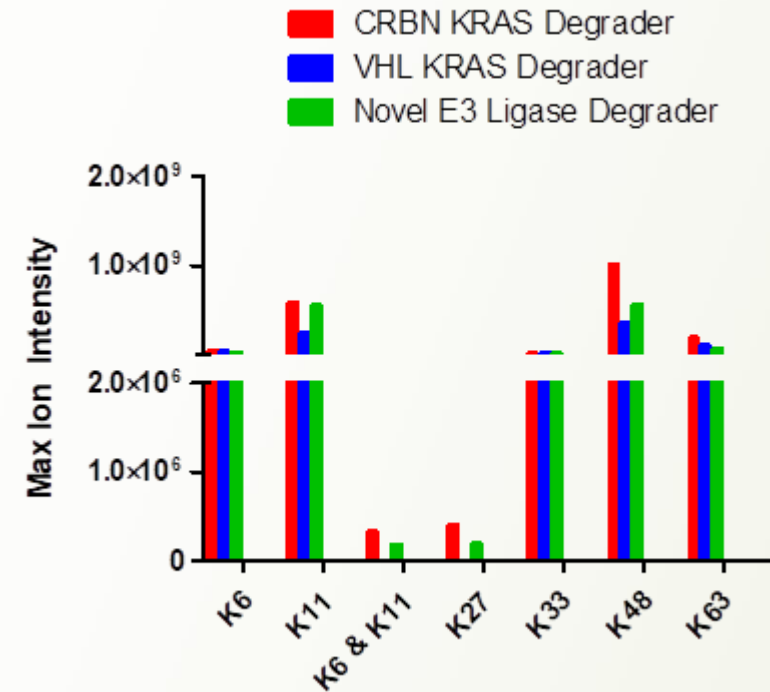


Monitoring Ubiquitination – Dose Response : changes in ubiquitination profiles of endogenous KRAS and subsequent degradation in H358 cells with changes in dose of both VHL and CRBN KRAS degraders. VHL and CRBN PROTACs designed with covalent ligands to engage KRAS G12C and successfully ubiquitinate and degrade with 3hrs of treatment between 30-100 nm.

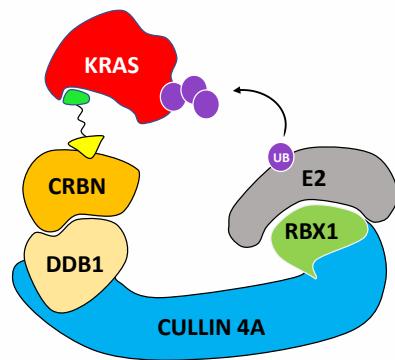
Mass Spectrometry Studies with KRAS Degraders

Profiling poly-ubiquitin chains, Comparative E3 Ligase Study – In vitro Ubiquitylation

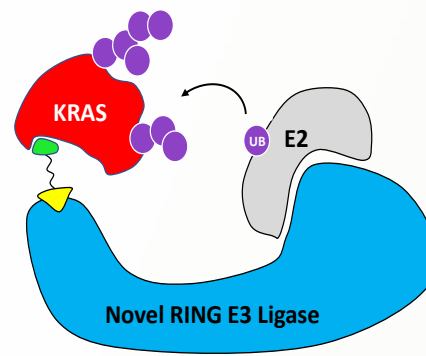
K-RAS Degradator / Ubiquitinated Peptide	K86	K103	K127	K146	K183
VHL based Degradator	Red	Orange	Green	Blue	Dark Green
CRBN based Degradator	Red	White	Blue	White	White
Novel E3 based Degradator	Confidential				



VHL E3 Ligase



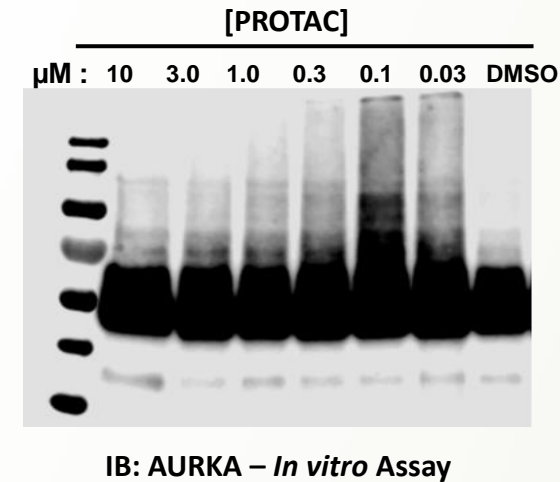
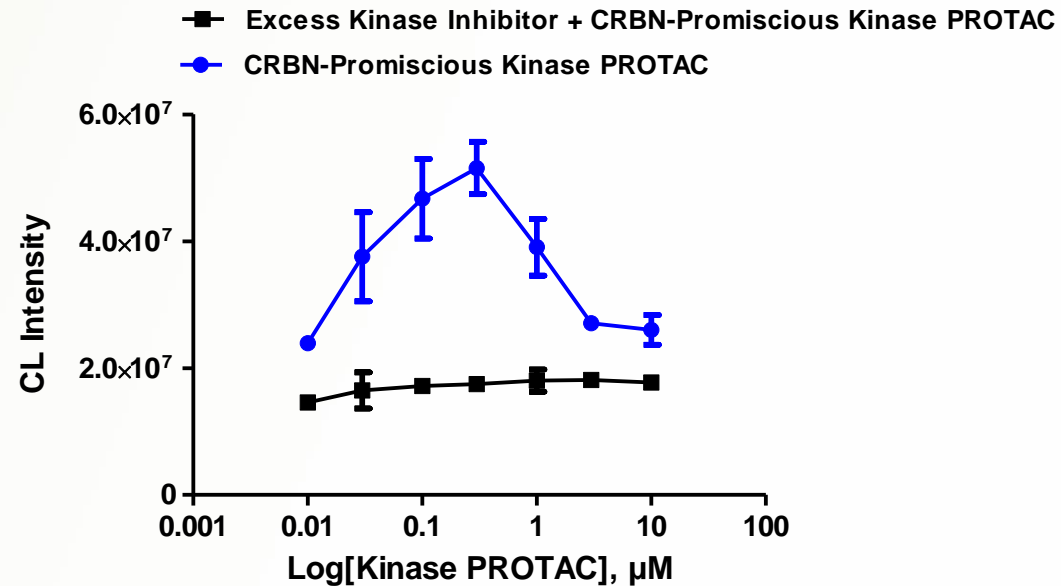
Cereblon E3 Ligase



Novel E3 Ligase

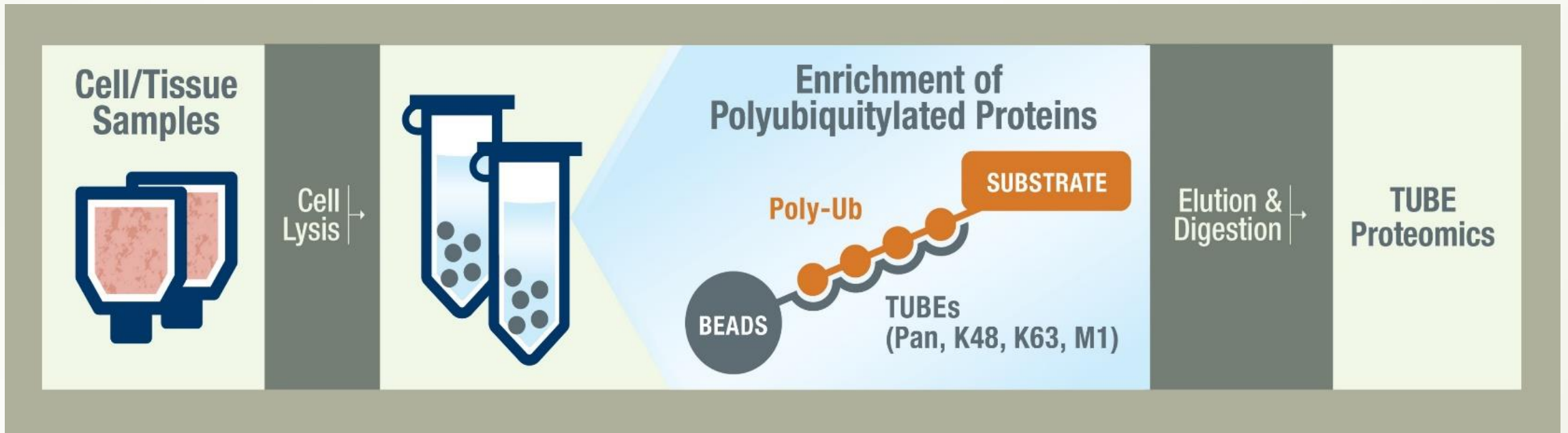
CRBN Multi-Kinase Degraders

Monitor PROTAC mediated *in vitro* Ternary Complex Formation and Ubiquitination - HTS



AURKA *in vitro* ubiquitination assays: recombinant AURKA ubiquitination was monitored as function a of cereblon PROTAC dose response. Excess kinase inhibitor competes with PROTAC interaction compromising ubiquitination of the target (see graph). A complementary gel-based assay to represent changes in poly-ubiquitination profiles mediated by PROTACs.

TUBE based Proteomics for Degraders

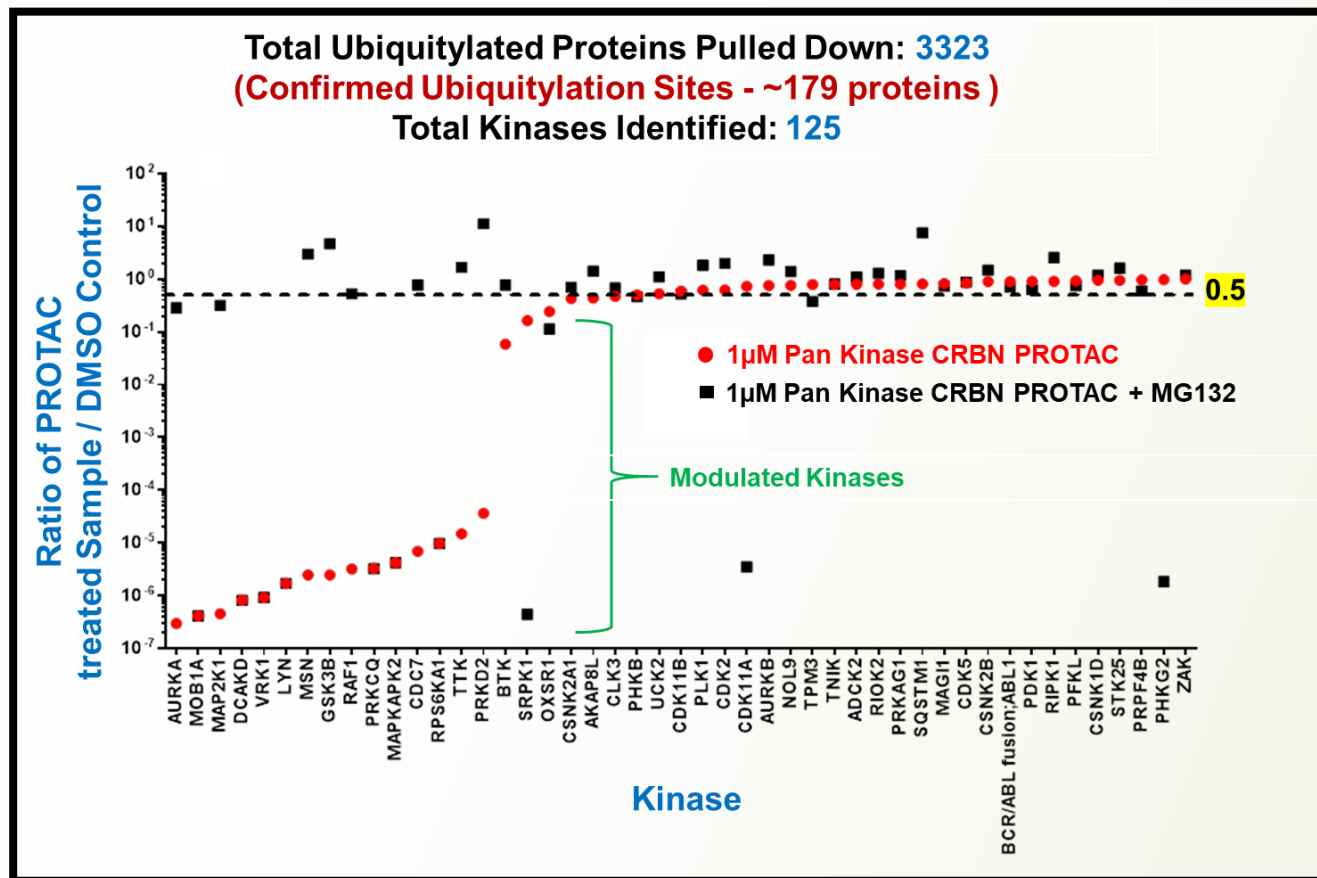


CRBN Multi-Kinase Degraders

TUBE Ubiquitomics to Study Degradable Kinome

Experiment: Cells treated w multi-kinase PROTAC. Ubiquitinated proteins were isolated by TUBE pulldown. Mass spec proteomic analysis was performed

Results: One batch of cells treated with PROTAC and the other batch with PROTAC plus proteasome inhibitor to block degradation. Data shows that many kinases are ubiquitinated by PROTAC and identified by TUBE-based proteomics



TUBE Based Platform to Analyze Mol Glues

- Rapidly discover and monitor Molecular Glues in a HTS on TUBE microtiter plates
- Monitor ubiquitination and degradation kinetics of **native** targets in vitro and in vivo
- TUBE-Based proteomics and discovery program allows to understand Mol Glue MOA
- Guiding Med Chem to establish rapid SAR

“Ub_{Max}” A better way to measure potency of Molecular Glues & PROTACs

TUBEs based Mol Glue Assays provides a link between ubiquitination and degradation

Future of Molecular Glues is Brighter

“Imposing our will with small molecules to orchestrate proximity by remote control is a powerful capability”

LifeSensors Leading the Way to UPS Drug Discovery

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