Mol Glues: The Future of TPD Drug Discovery

Unleashing the Potential of E3 Ligases and TUBE Technology

LifeSensors Inc.

271 Great Valley Parkway

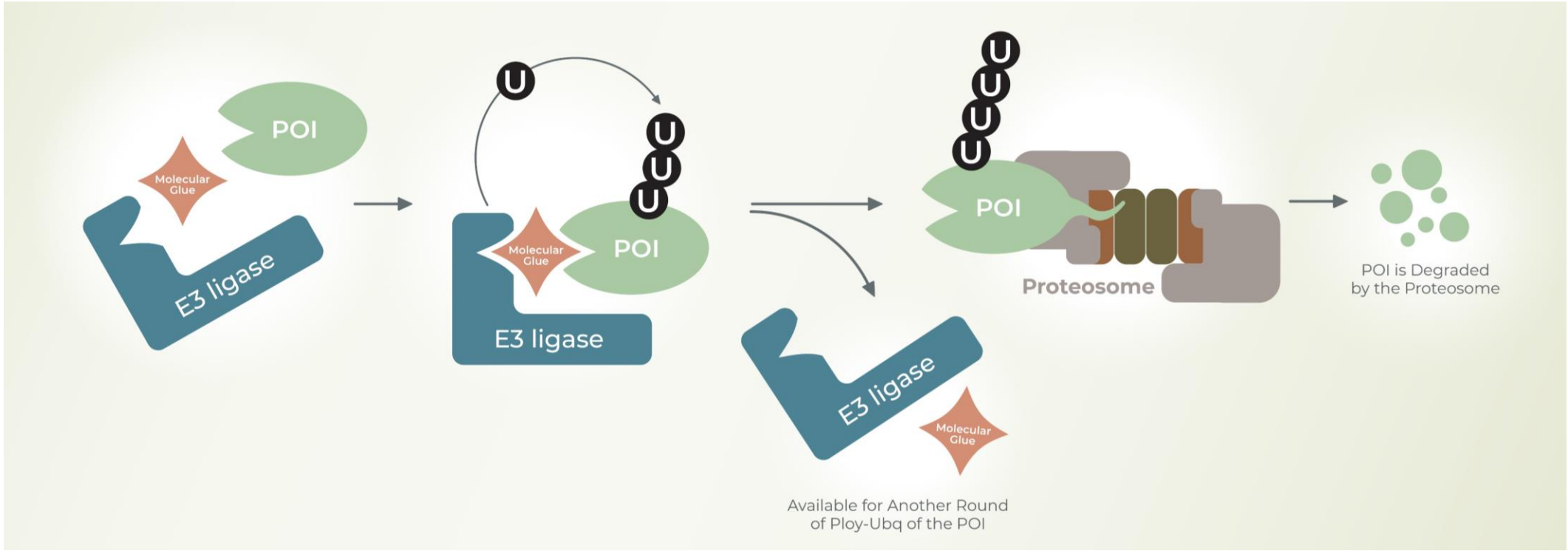
Malvern PA 19355

Phone: 610-644-8845 x 310

bd@lifesensors.com

www.lifesensors.com

Molecular Glues: Monovalent Targeted Degraders



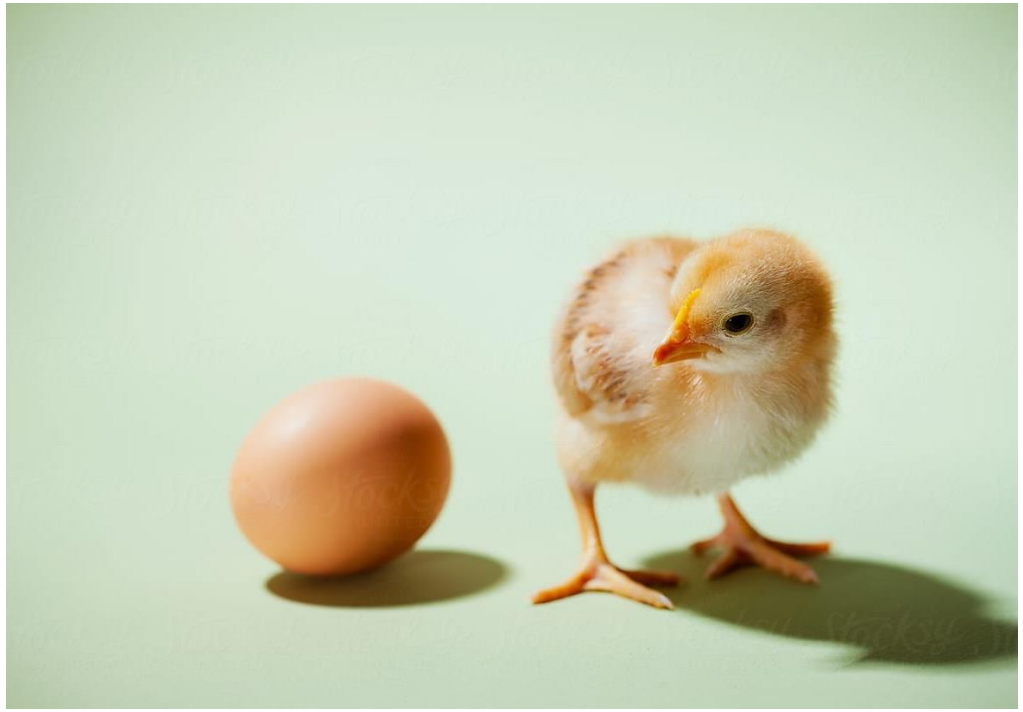
It Took 60 Years, Tragedies and Serendipities to Discover Thalidomide Molecular Glues

Vast Applications of Mol Glues in Drug Discovery

Small molecules that selectively change confirmation of proteins to:

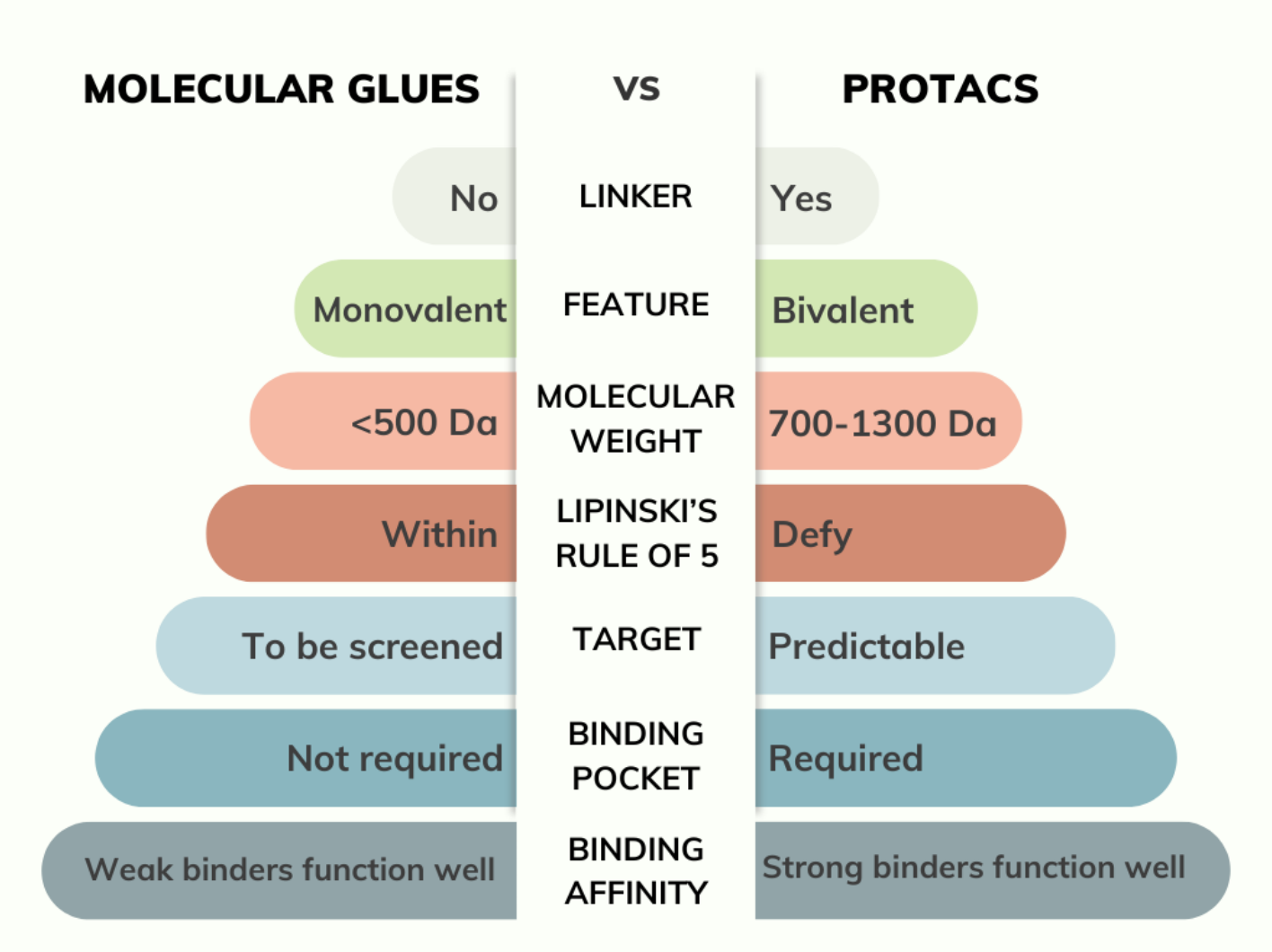
- **Bind to E3 ligases and promote ubiquitination of neo-substrates**
- **Bind to proteins, in proximity of E3 ligases, to promote ubiquitination of target proteins**
- **MGs may promote compartmentalization, loss of function or stabilization of target proteins**

Rational Way to Screen for Molecular Glues

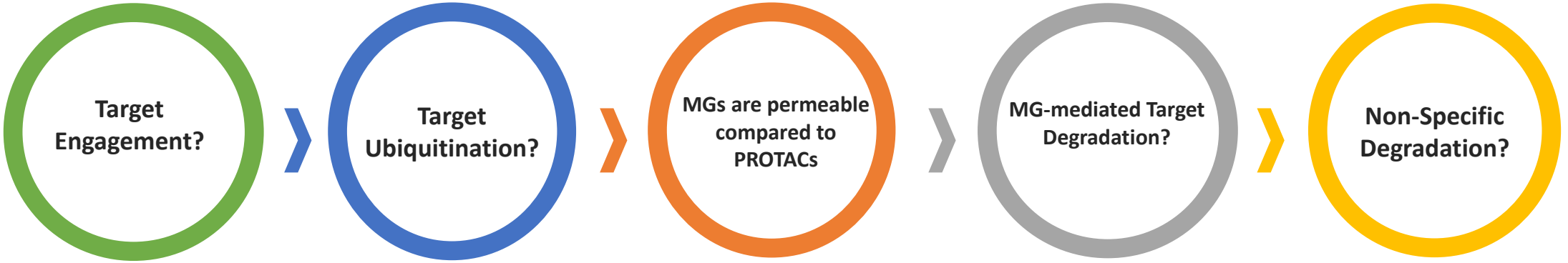


Monitor Ubiquitylation First

Differentiating Molecular Glues and Traditional PROTACs



Current Tools for Molecular Glue Research



Biophysical Screening

- ✓ FP
- ✓ TR-FRET
- ✓ SPR
- ✓ DSF
- ✓ Modeling

Biochemical/Cellular Screening

- ✓ Western blotting
- ✓ LifeSensors *In vitro* Ubiquitination Assays
- ✓ LifeSensors TUBE-embedded microtiter plated based assay

Cellular Permeability / Target Engagement

- ✓ Promega NanoBRET fusion tag assays

Monitor Degradation *in vivo*

- ✓ Endpoint -Western blot
- ✓ Kinetic – Reporter gene assays (HiBiT, GFP, nanoLuc etc.)
- ✓ Kinetic – LifeSensors Degradation assays
- ✓ LifeSensors TUBE-embedded plated based assay

Specificity

Challenges

- Does not represent True MG function

Challenges

- Antibody epitope screening

Challenges

- Extensive Optimization – cell line compatibility

Challenges

- Reporter Tag artifacts
- WBs - Unreliable

Challenges

- Time consuming

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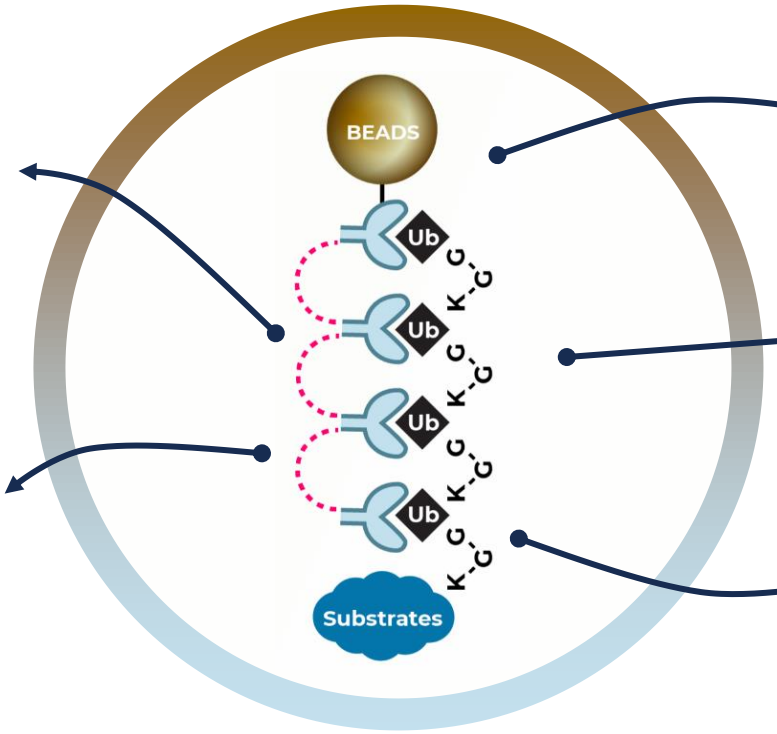
TANDEM UBIQUITIN BINDING ENTITIES

TUBEs: A Versatile Tool for Mol Glue Drug Discovery

Ubiquitin Binding Domains

Engineered for Chain Selectivity



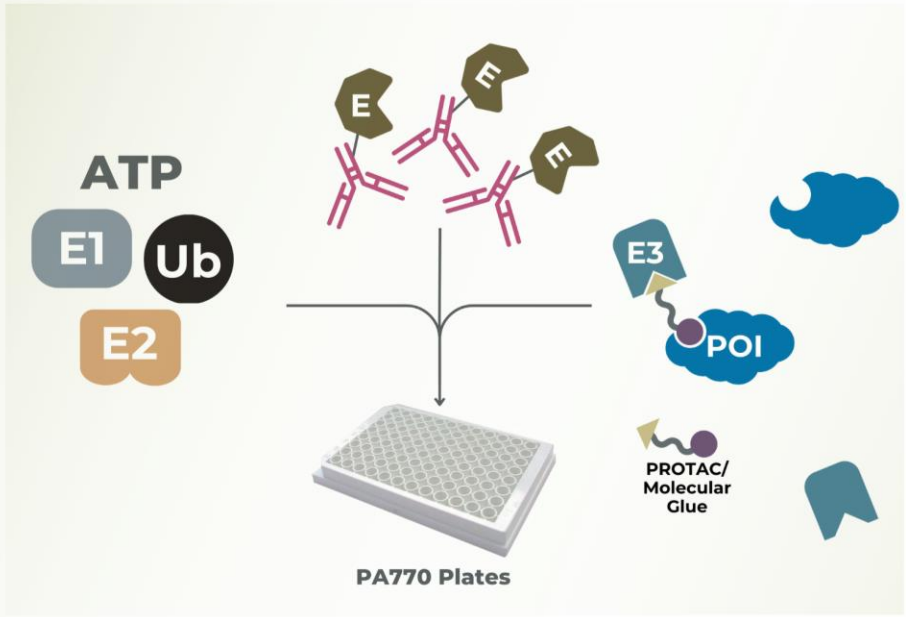
Versatile applications

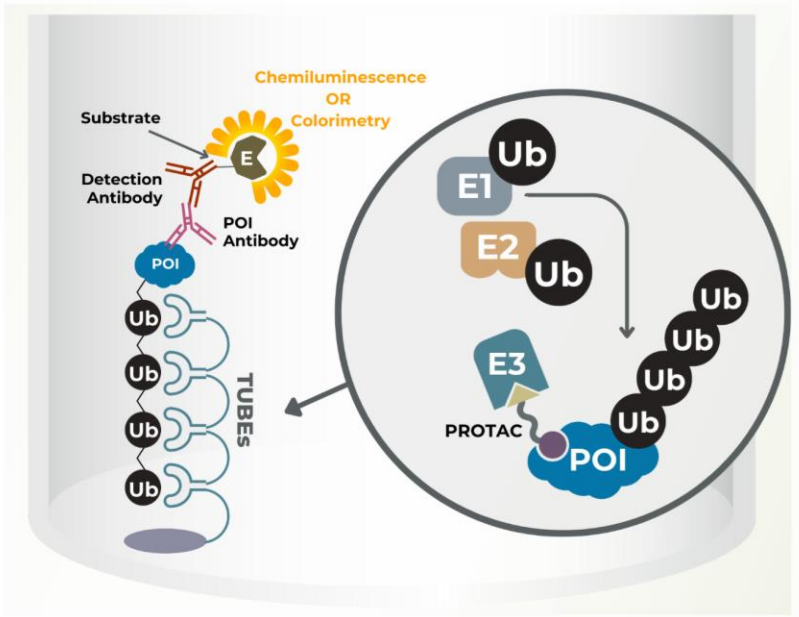
Sub-nanomolar affinity binding

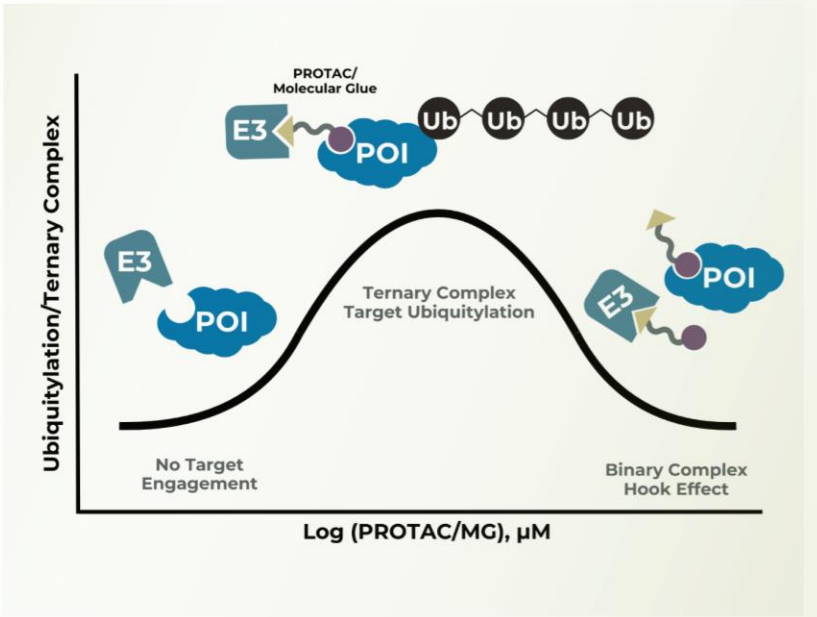
Protection against intrinsic DUBs

Microtiter Plate Embedded TUBE- In Vitro MGs Screening Platform

Monitor Molecular Glue Mediated Ubiquitination Capture on Plates







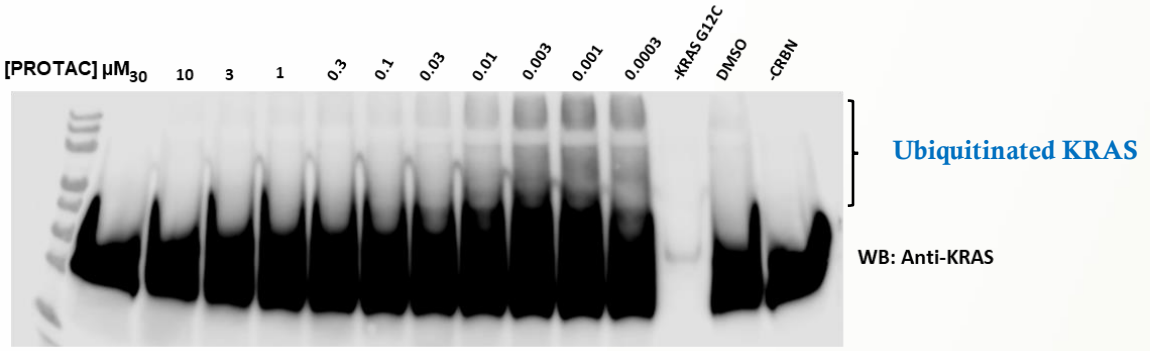
TUBE capture & PROTAC/MG mediated ubiquitination of POI detection

CRBN & VHL K-RAS Degraders

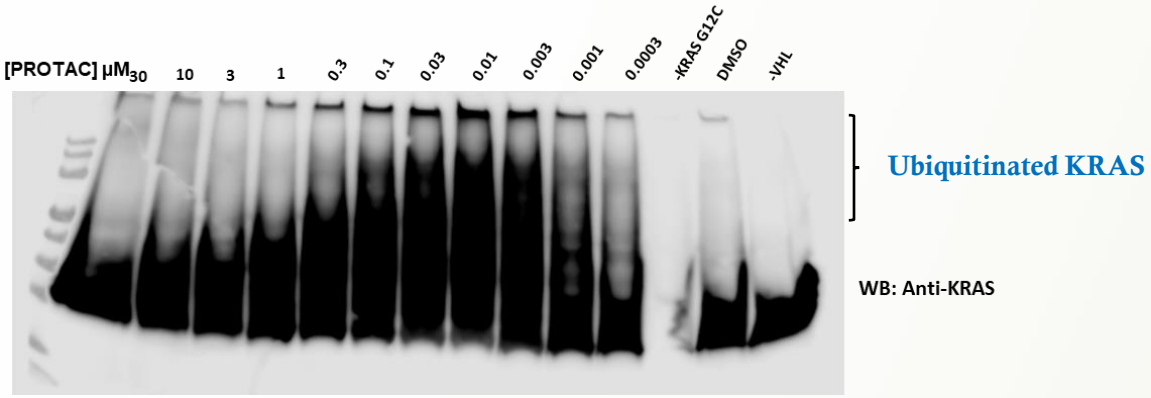
Monitor Molecular Glue Mediated Ubiquitination of KRAS G12C vs G12D in vitro, – Western blotting

Please note ligase and PROTAC mediated ubiquitination increases the mol wt of K-RAS on gels

CRBN KRAS G12C Degrader

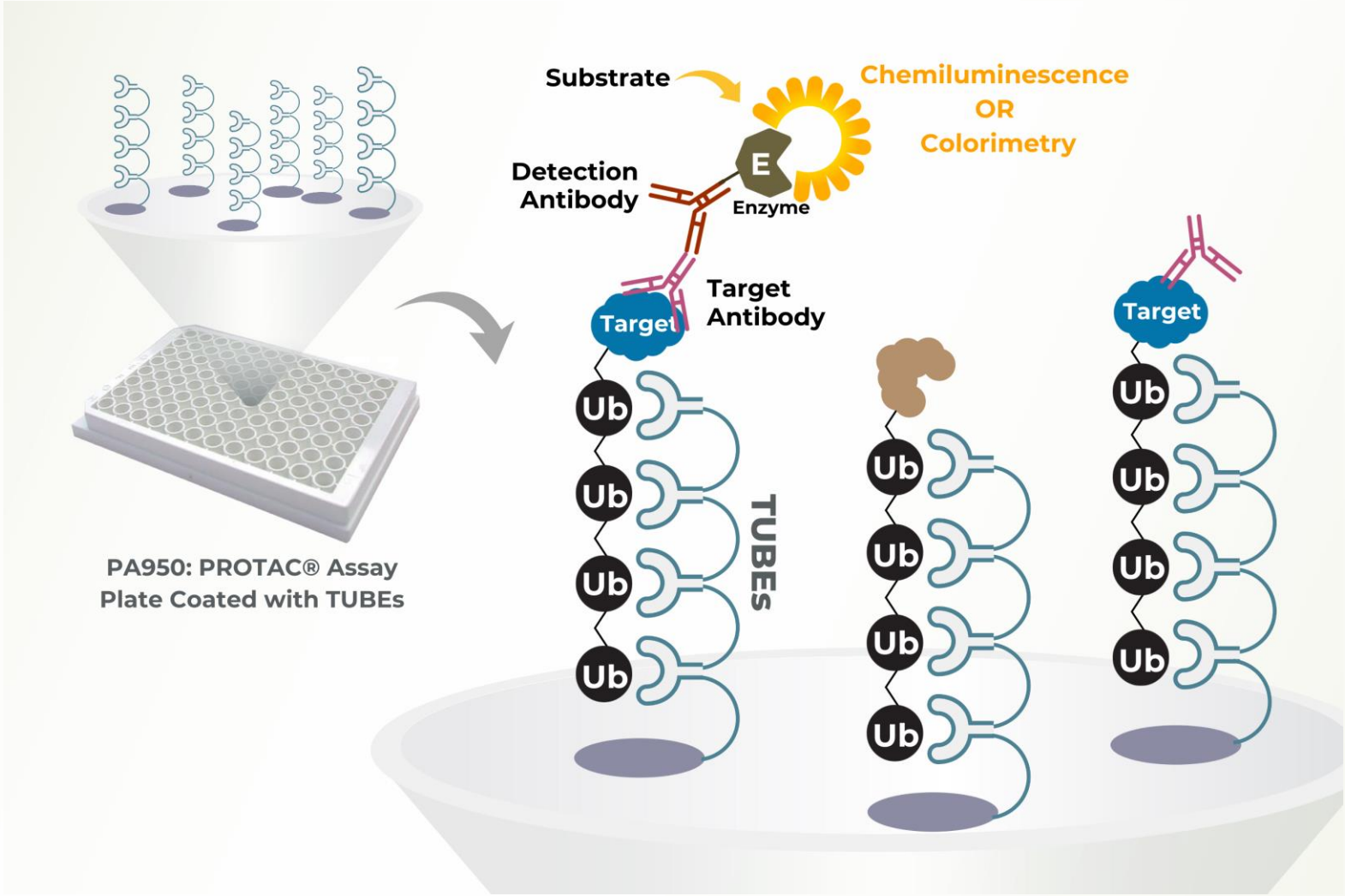


VHL KRAS G12C Degrader (LC2)



Ubiquitination Assays– Cell Based Assays

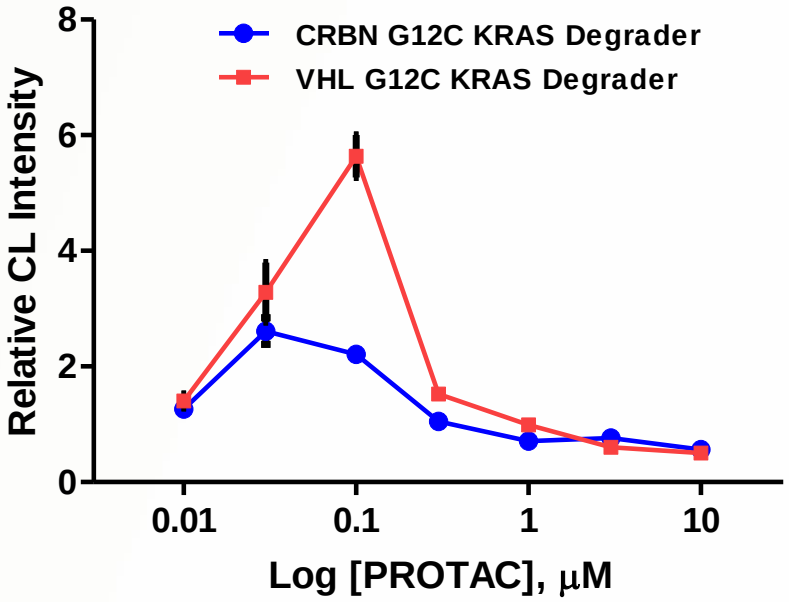
Monitor Molecular Glue Mediated Ubiquitylation and Degradation by HTS on **TUBE Microtiter plates**

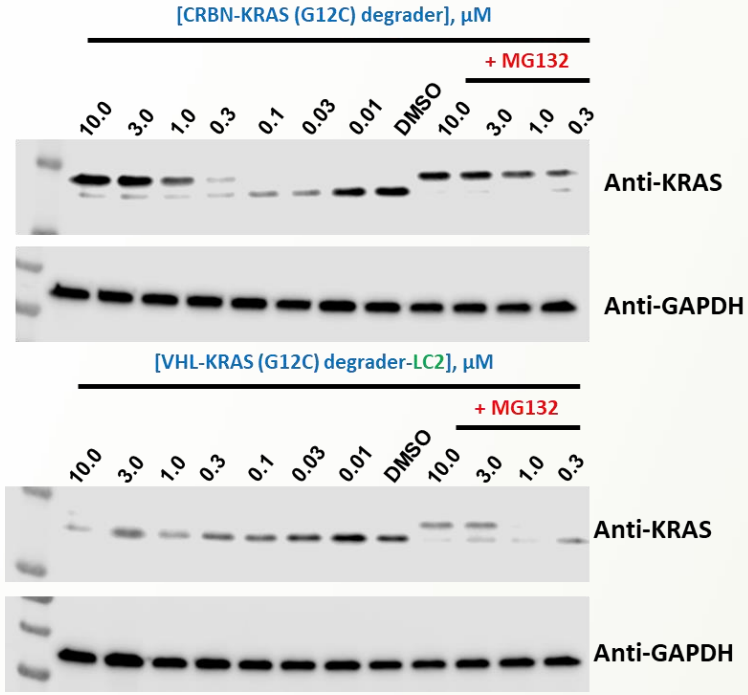


CRBN & VHL KRAS Degraders

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.

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

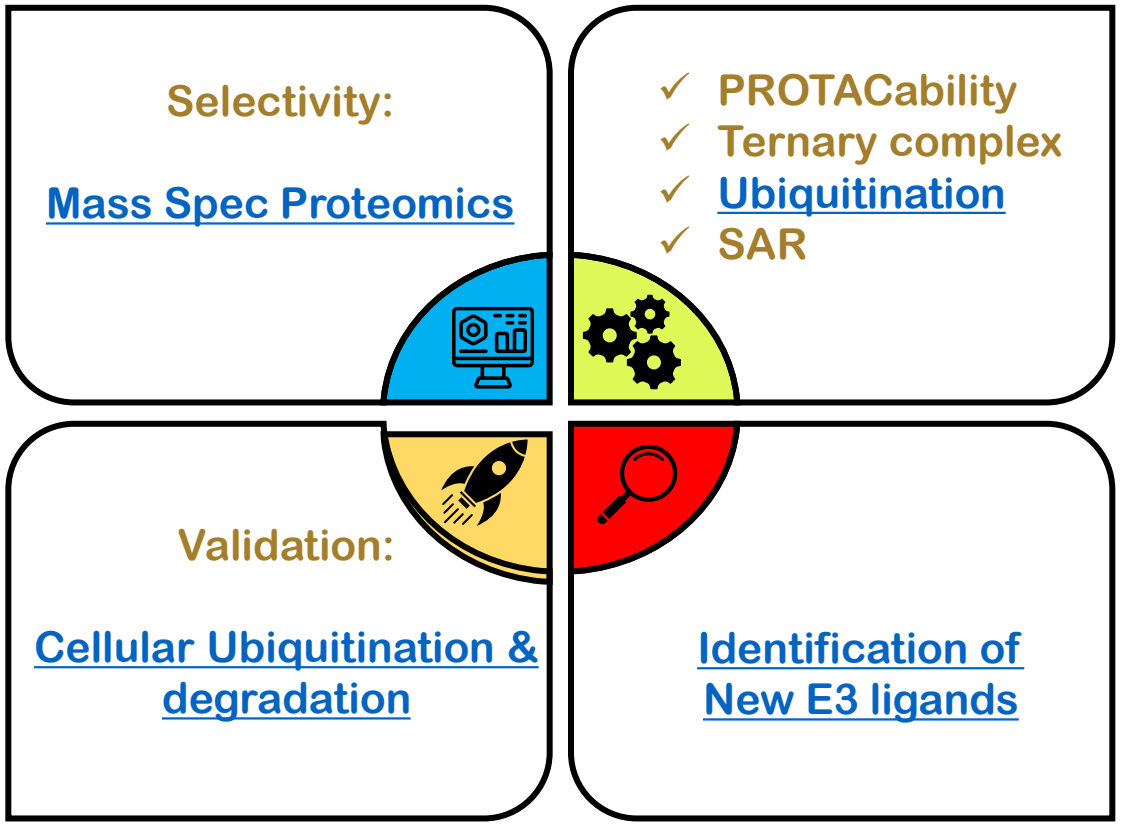
- **“Ub_{Max}” A better way to measure potency of Molecular Glues & PROTACs**

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 Approaches for Targeted Protein Degradation



Contact Us!

We are your partner for Mol Glue drug discovery

Contact Information

Research & Product Inquiries	**R&D**	**info@lifesensors.com**	**610-644-8845 (ext 339)**
Custom Service & Assays	**BD**	**bd@lifesensors.com**	**610-644-8845 (ext 354)**