E3 Ligase Profiling & Screening

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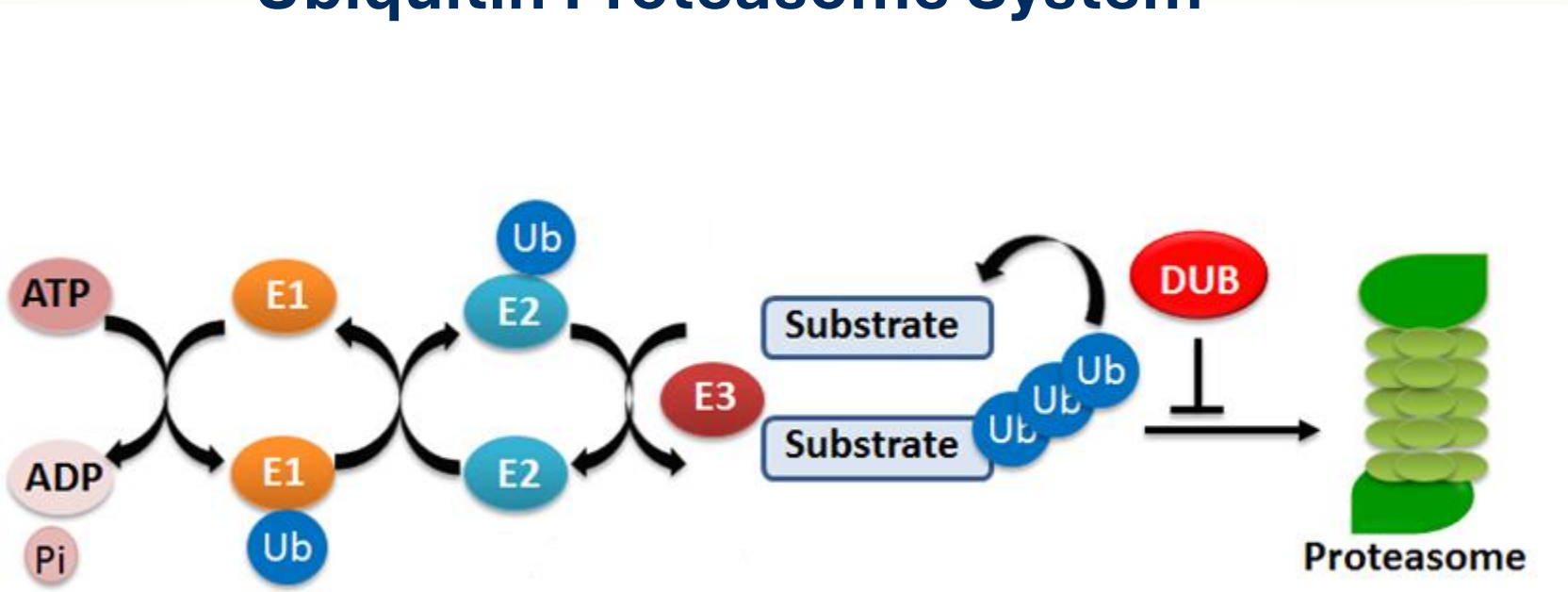
LifeSensors

- Leading Biotech in Ubiquitin Proteasome System (UPS) Drug Discovery
- ~500 Products: Proteins, Ubiquitin Affinity Reagents (TUBEs), Inhibitors, Assays, Kits and Proprietary SUMO Protein Expression Systems
- Drug Discovery, UPS and PROTAC Screening Services
- Profiling Compounds Against Ubiquitin Ligases and De-Ubiquitinases (DUBs)
- Custom Assay Development and Collaborative Research

E3 Ligase Drug Discovery Capabilities

- Expressed and purified ~40 biologically active E3 ligases
- Developed 30 different assays for E3 ligases (auto and substrate ubiquitylation)
- Ability to screen ~650,000 compounds
- Enzyme selectivity panels and compound profiling
- Determine compound MOA, cellular and target tissue PD markers
- Enabling technologies based on TUBE applications

Ubiquitin Proteasome System



E1 – Ubiquitin activating enzyme

Requires ATP to attach Ub to E1

E2 – Ubiquitin conjugating enzyme

Transfers Ub from E1 to E3

E3 – Ubiquitin ligases

Transfers Ub to self or substrate

Forms mono-Ub or poly-Ub chains

DUB – Deubiquitinase

Removes mono-Ub or poly-Ub chains

Proteasome – Degrades ubiquitylated proteins

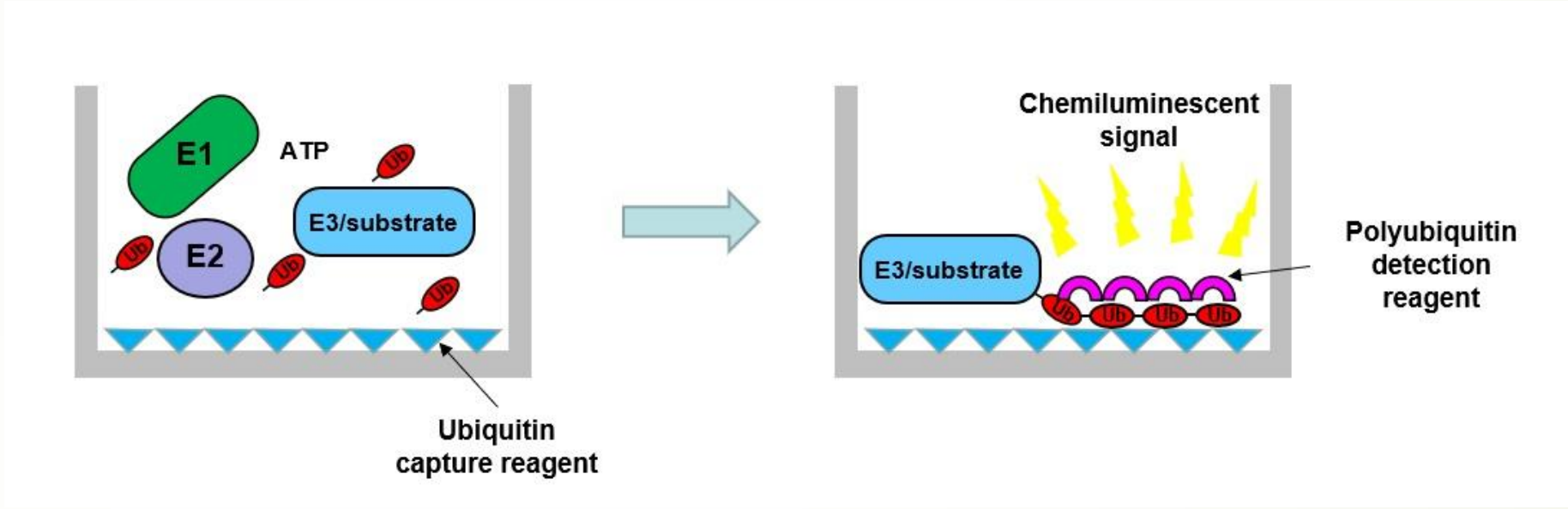
E3 Ligase Drug Screening Overview

- **Step One: Assay development, optimization and HTS**
  - E3 ELISA Assay
  - TR-FRET E3 Assay
- **Step Two: Hit-to-lead optimization**
  - Working with medicinal chemistry team
  - Selectivity panel, compound profiling
- **Step Three: Confirm hits in cellular assays**
  - UbiQuant S assay (ELISA / AlphaLISA)
  - UbiTest (Immunoblot-based assay)

Step One: Assay Development, Optimization and HTS

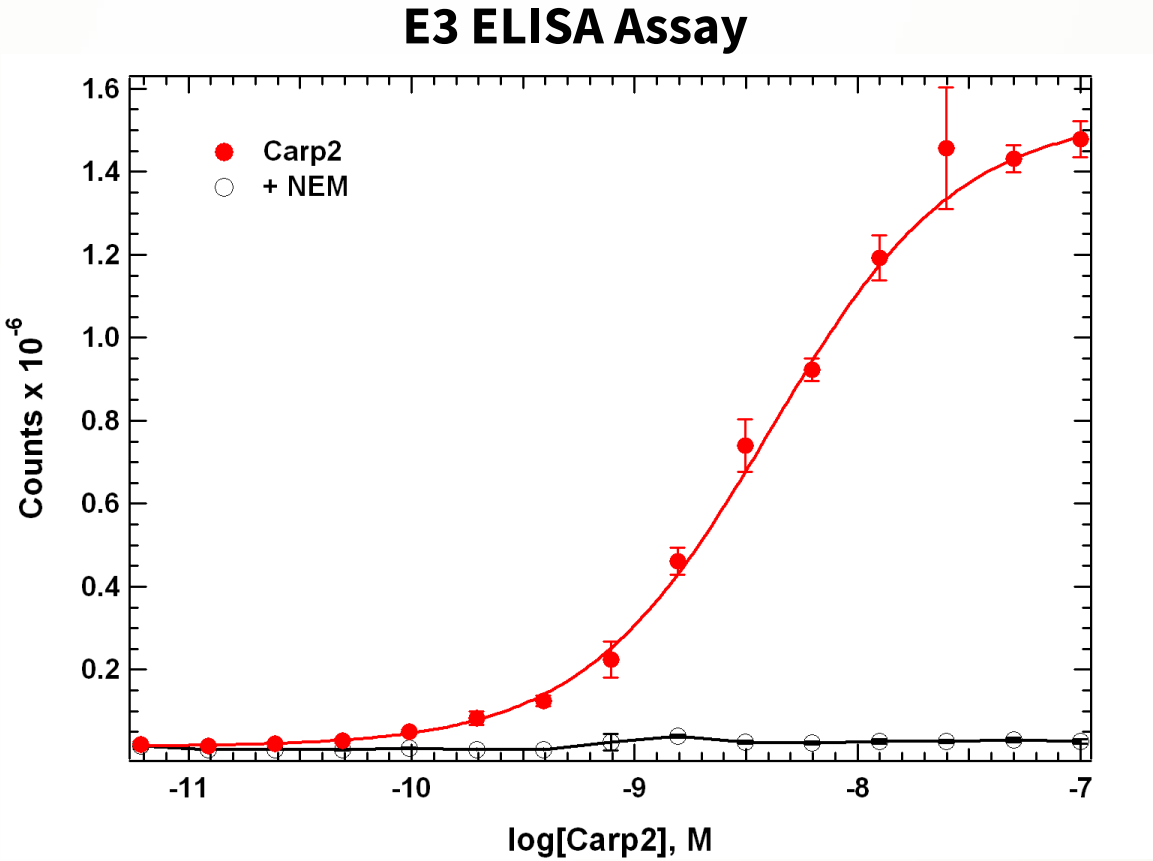
E3 Ligase ELISA Assays

Quantification of E3 ubiquitin ligase activity, employs a proprietary TUBE reagent to capture polyubiquitin chains formed in an E3 ligase dependent manner



The polyubiquitylated substrate is detected using HRP-conjugated TUBEs. The chemiluminescent signal can be followed over time in a homogenous, high-throughput format, making it ideal for small-molecule screening.

Example of E3 Ligase ELISA Assay

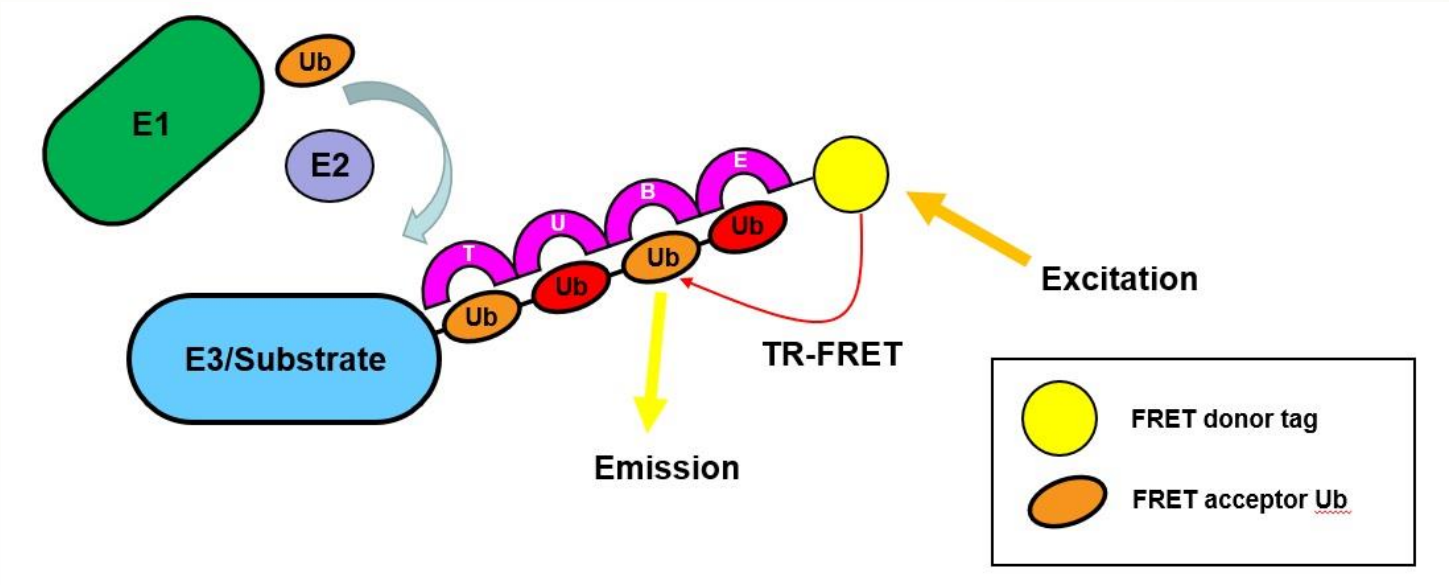


Dose response of CARP2 with ubiquitin E3 ligase activity assay

Step One: Assay Development, Optimization and HTS

TR-FRET E3 Ligase Assay

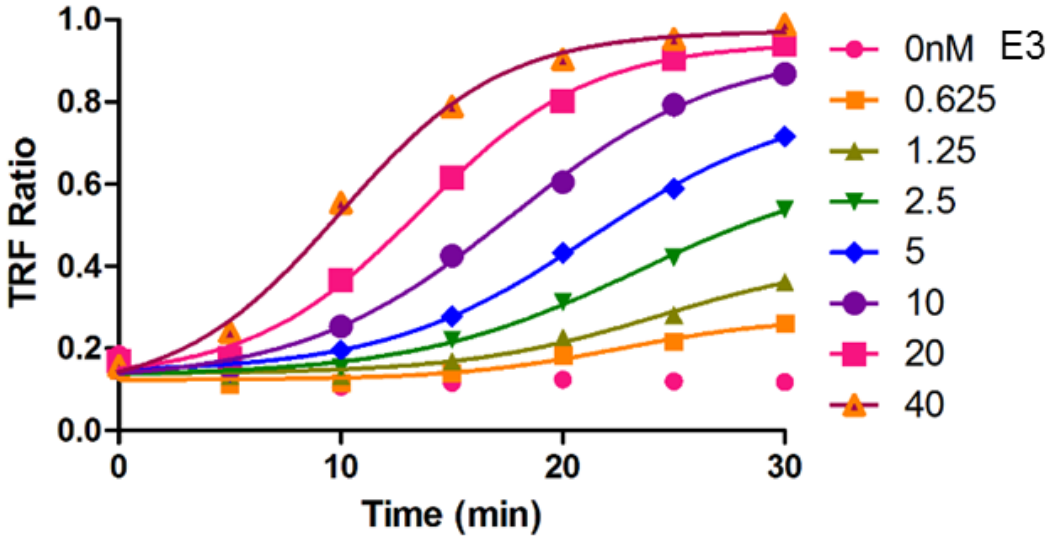
Fluorescence-based high-throughput assay system for screening compound libraries against E3 ligase activity



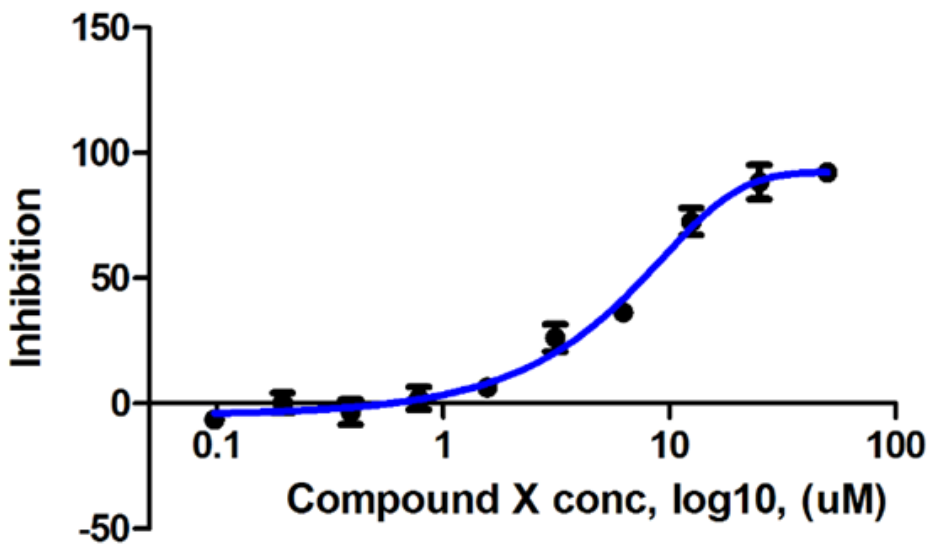
The **TR-FRET E3 Assay** involves terbium-labeled TUBEs that bind to fluorescein-labelled polyubiquitin chains synthesized by the target E3 ligase. Terbium and fluorescein are a FRET pair, so polyubiquitin chains containing fluorescein-labeled ubiquitin yield a FRET signal when bound by a terbium-TUBE. This signal can be monitored over time in a homogenous, high-throughput format, making it ideal for small-molecule screening.

Example of TR-FRET E3 Ligase Assay

E3 Titration
Protein X Ubiquitination



Protein X Ubiquitination
Inhibition



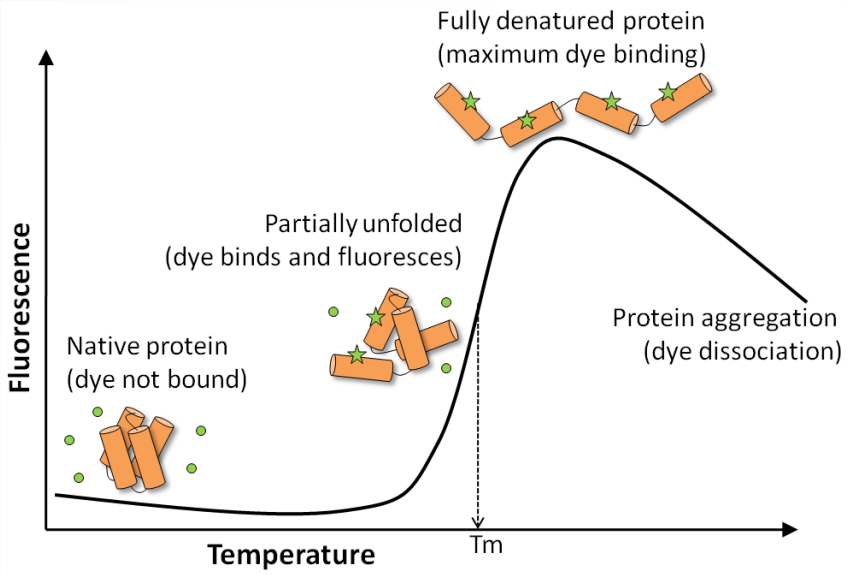
E3 TR-FRET assay and inhibitor dose response curve
Protein X was used as a substrate for this E3 ligase. After initial TR-FRET high-throughput screening, selected comppounds were used to determine IC_{50} by titration assay.

Step Two: Hit-to-lead optimization

Validation Assays

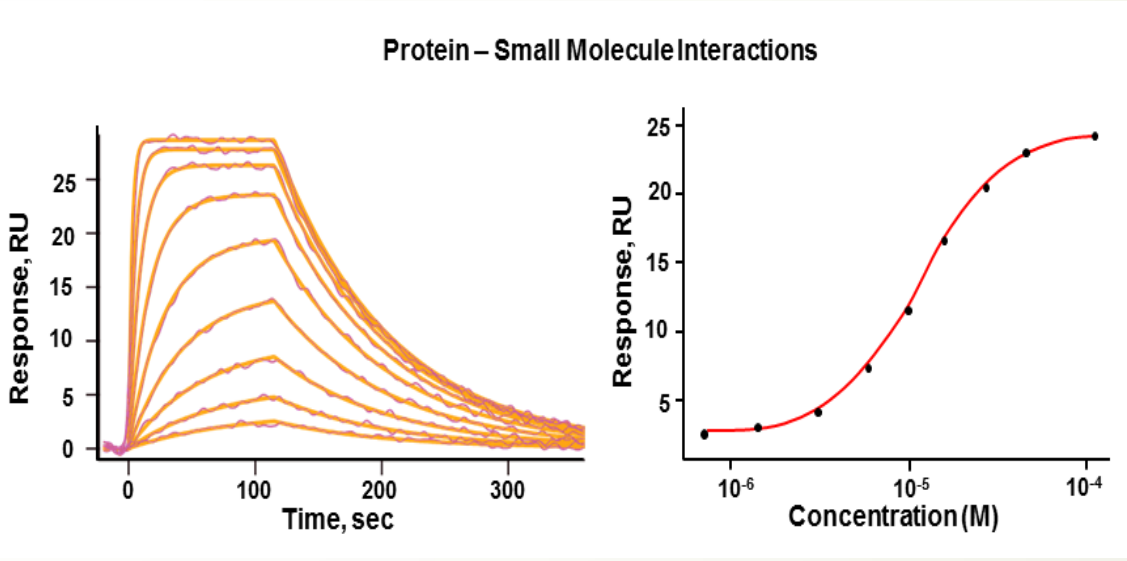
Thermal Shift Assay

HTS assay to detect compound binding to a target



Surface Plasma Resonance

Determination of a small molecule affinity to a target



Step Two: Hit-to-lead optimization

Selectivity Assays

E3 Ligase Panel	Representative E3s
Panel I (5 E3 ligases)	CRBN, CARP2, gp78, CHIP, Nedd4L
Panel II (10 E3 ligases)	CRBN, VHL, HDM2, cIAP2, CARP2, gp78, CHIP, Nedd4L, Praja1, Cbl-b
Panel III (29 E3 ligases, includes E3 from panel I as well)	CRBN, VHL, Hdm2, RNF4, CARP2, TRIM32, TRIM47, Cbl-b, c-Cbl, cIAP2, IDOL, SIAH, MURF1, MURF2, MURF3, Praja1, TRAF6, Parkin, E6-AP, Itch, Nedd4L, WWP1, WWP2, MARCH5, Hrd1, gp78, CHIP, RNF114, Nedd4

Each ligase assay has been validated in TR-FRET assays regarding E2 pairing. LifeSensors profiles inhibitory or activation properties of every compound in Panel I followed by Panel II.

Step Two: Hit-to-lead optimization

Mechanistic Validation Assays

Secondary screens to deconvolute hits from E3 screening

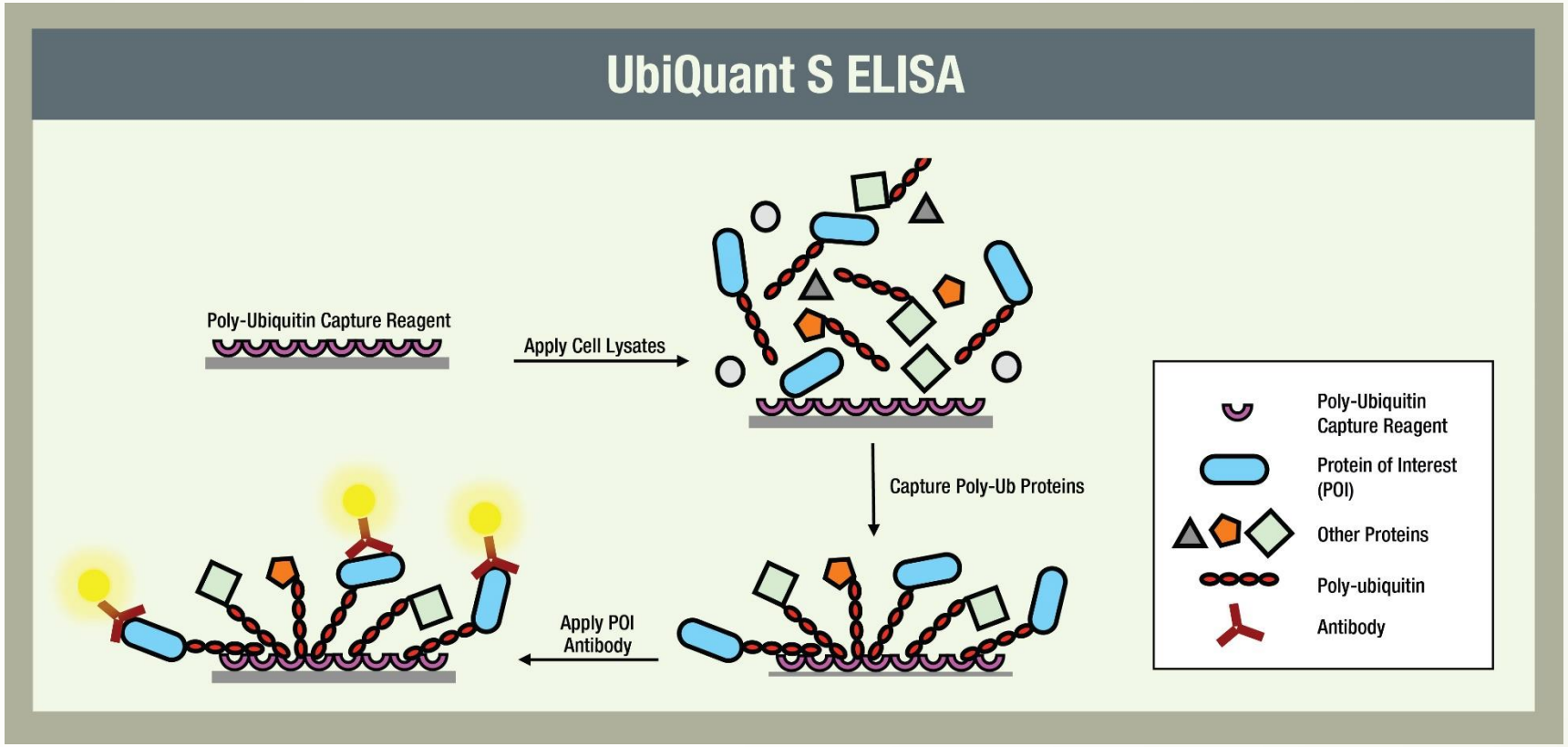
(eliminating compounds that affect E1-E2 conjugation)

- **E3 Lite** - Measures E3 activity
- **E1 Lite** - Measures E1 activity
- **E1/E2 transfer** - Measures transfer between E1 to E2
- **E2 Profiling and Selection** - Finds the best E2 for your E3

Step Three: Confirm hits in cellular assays

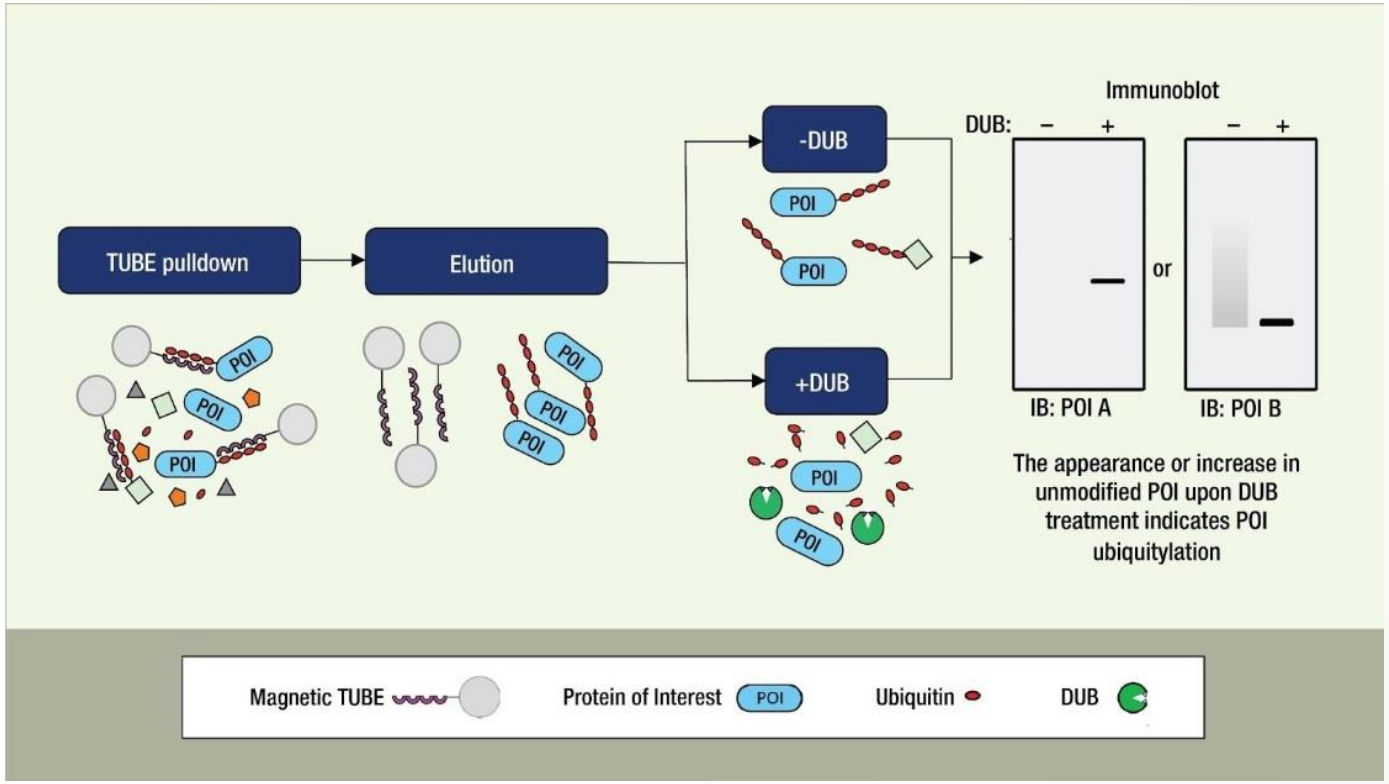
UbiQuant S ELISA Assay

Enables accurate determination of substrate (POI) ubiquitylation for monitoring the effects of various treatments on patterns of cellular substrate ubiquitylation



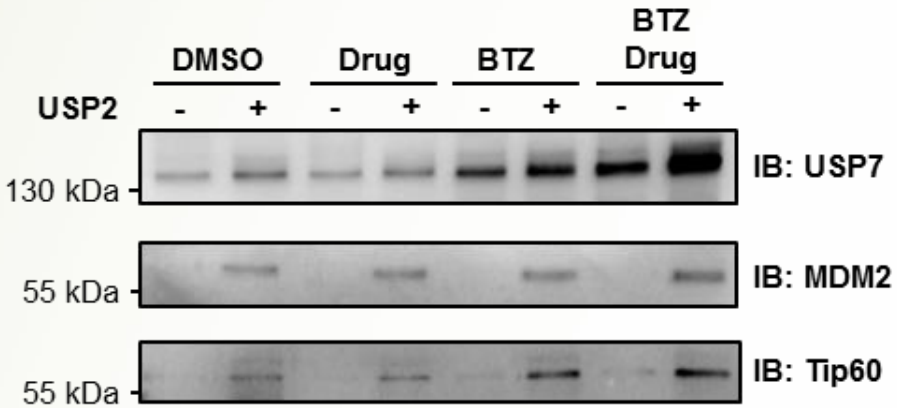
Step Three: Confirm hits in cellular assays

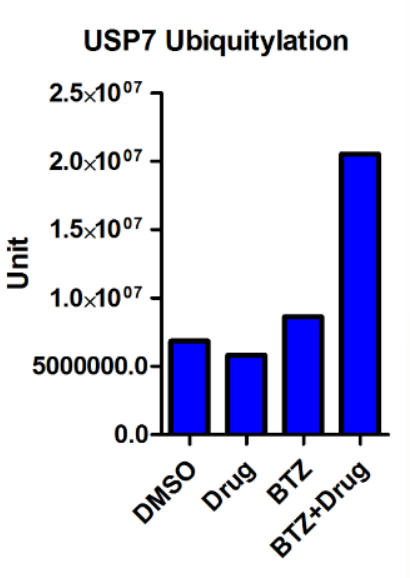
UbiTest: Assay to measure ubiquitylation of POI in cells

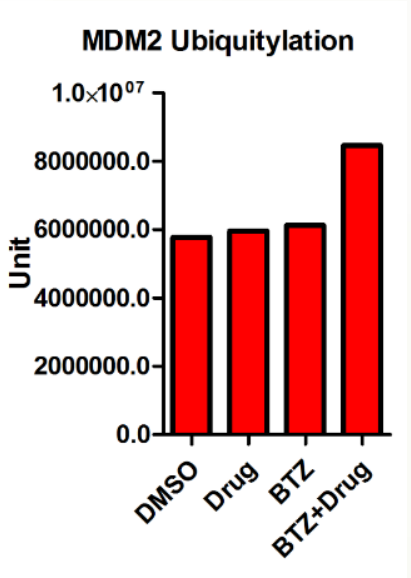


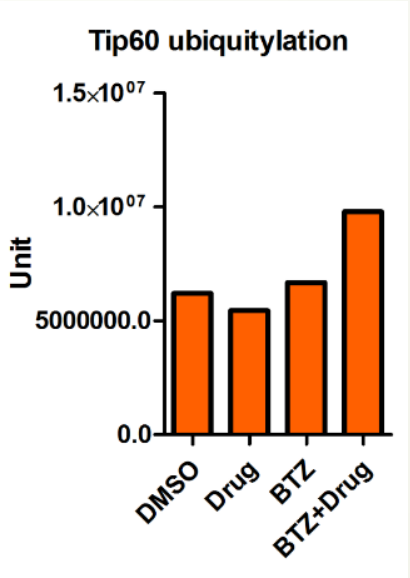
UbiTest – a TUBE-based pull-down method that isolates total cellular ubiquitylated proteins. Subsequently, samples are treated with pan-selective DUBs to remove polyubiquitin chains. The target protein is identified by its native molecular weight and analyzed and quantified by immunoblotting. UbiTest is one of the most sensitive methods available to quantify ubiquitylation levels of proteins in vivo.

Example of UbiTest Assay









Determine endogenous target protein ubiquitylation using UbiTest

Jurkat cells were treated with indicated compounds and lysed in RIPA buffer. Anti-Ub TUBE1 agarose resin was added for pull down total polyubiquitylated proteins and then elution was incubated with DUB. Immunoblot (left) of the assay is shown and quantitation (right) of the bands showed increased signal of USP7, MDM2, Tip60 after DUB treatment indicating they are polyubiquitylated.

Identification E3 ligase modulators for Clients:

Example #1

- **E3 ligase X: Assay development, validation and HTS (include timeline)**

  50K small molecule library screen using TR-FRET E3 Assay

  X number of primary hits, Z' >0.5 (maybe show a scatter plot of screen)

  X number of confirmed hits with selectivity (maybe show a table for selectivity)

  X number of compounds with IC50s sub micromolar to nM (show representative graphs of dose response curves)

- **Step Two: Hit-to-lead optimization**

  Hit expansion (with medicinal chemistry team)

  Extended selectivity panel, compound profiling

- **Step Three: Confirm hits in cellular assays**

  X number of hits transferred to client for cellular validation (in progress)

Identification E3 ligase modulators for Clients:

Example #2

- **E3 ligase X: Assay development, validation and HTS (include timeline)**

  Client's compounds screened using TR-FRET E3 Assay

  X number of confirmed compounds with selectivity

  X number of compounds with IC50s sub micromolar to nM

- **Step Two: Hit-to-lead optimization**

  Hit expansion (with medicinal chemistry team)

  Extended selectivity panel, compound profiling

- **Step Three: Confirm hits in cellular assays**

  In progress

E3 Ligase Screening & Profiling Services

- Help customer discover E3 ligase ligands, inhibitors and activators
  - Express & purify biologically active E3 Ligases and substrates
  - Develop and optimize HTS assay for E3 ligase
  - Screen in house libraries or customer libraries at LifeSensors
  - Confirmation and counter screening to eliminate off-target compounds
  - Biophysical and biochemical assay development for target engagement
- Cell-based assays to determine target engagement by compound
- All IP and data belong to the customer
- Work performed under CDA and Master Service Agreement
- Fee for service model, defined milestone-based agreement

Contact Us!

We are your partner for E3 Ligase drug discovery

Contact Information

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