

# DUB Screening & Profiling

## LifeSensors

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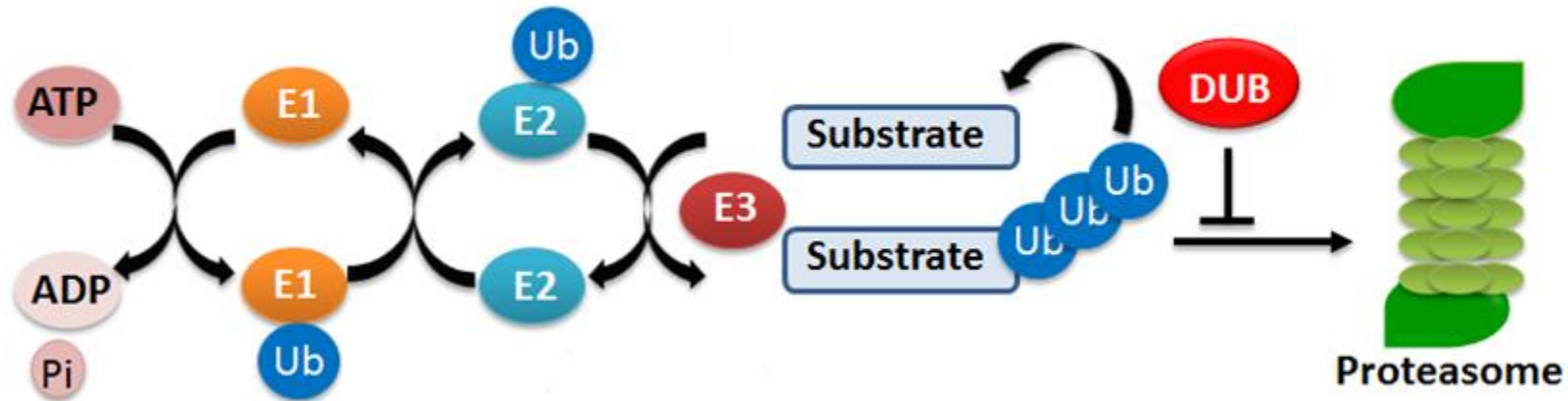
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# LifeSensors

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

# 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

# DUB-based Drug Discovery Capabilities

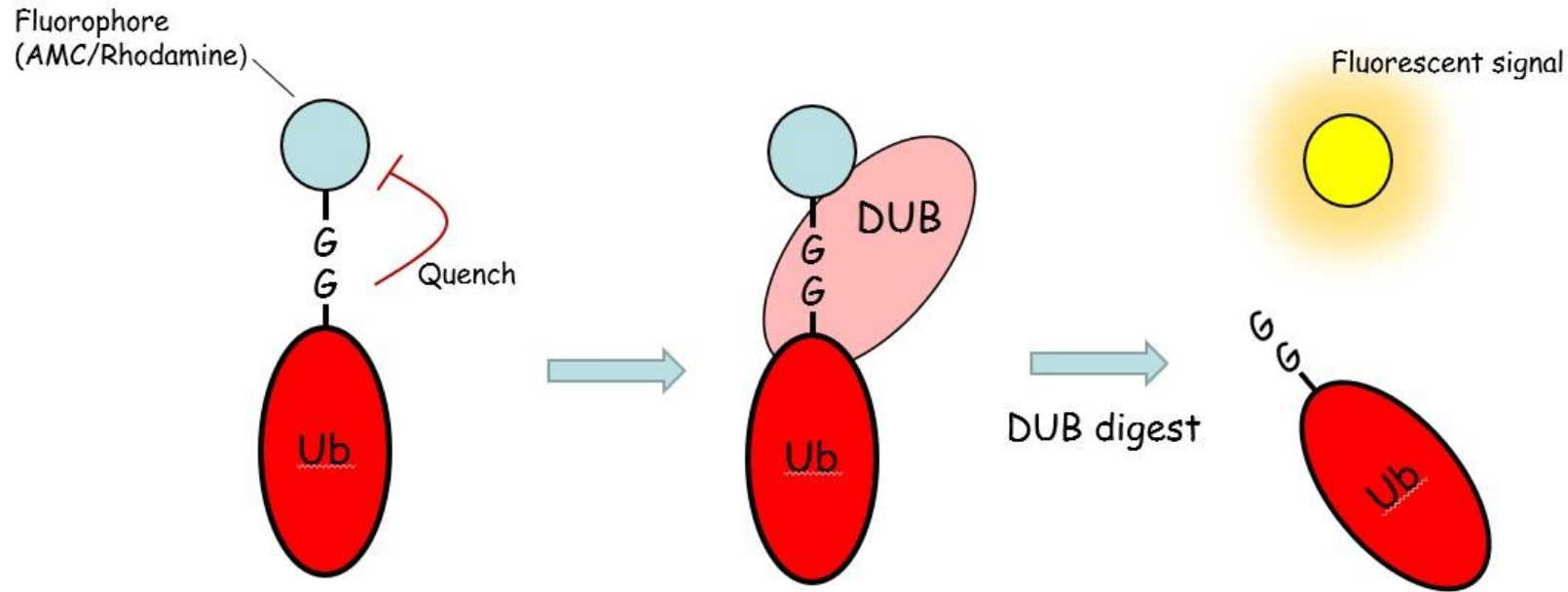
- ~40 biologically active DUBs
- Developed ~25 DUB assays for a variety of HTS and validation strategies
- Ability to screen ~500,000 compounds
- Enzyme selectivity panels and compound profiling
- Determine compound MOA, cellular and target tissue PD markers
- Enabling technologies based on TUBE applications

# LifeSensors DUBs

DUB Family	Representative DUBs
USP	USP2, USP5, USP7, USP8, USP15, USP20, USP21, USP30, USP34, USP47, USP51
OTU	OTUB1, Cezanne,
UCH	UCHL1, UCHL3, UCHL5
Viral & Bacterial	PLPro, PLP2, Ssel
MJD	Ataxin 3, JosD1
JAMM	AMSH
DeSUMOylase	Ulp1, SENP2, SENP6
DeNEDDylase	Den1
MINDY	MINDY1

# Step One: DUB Assay Development, Optimization and HTS

## DUB HTS and Validation Assays

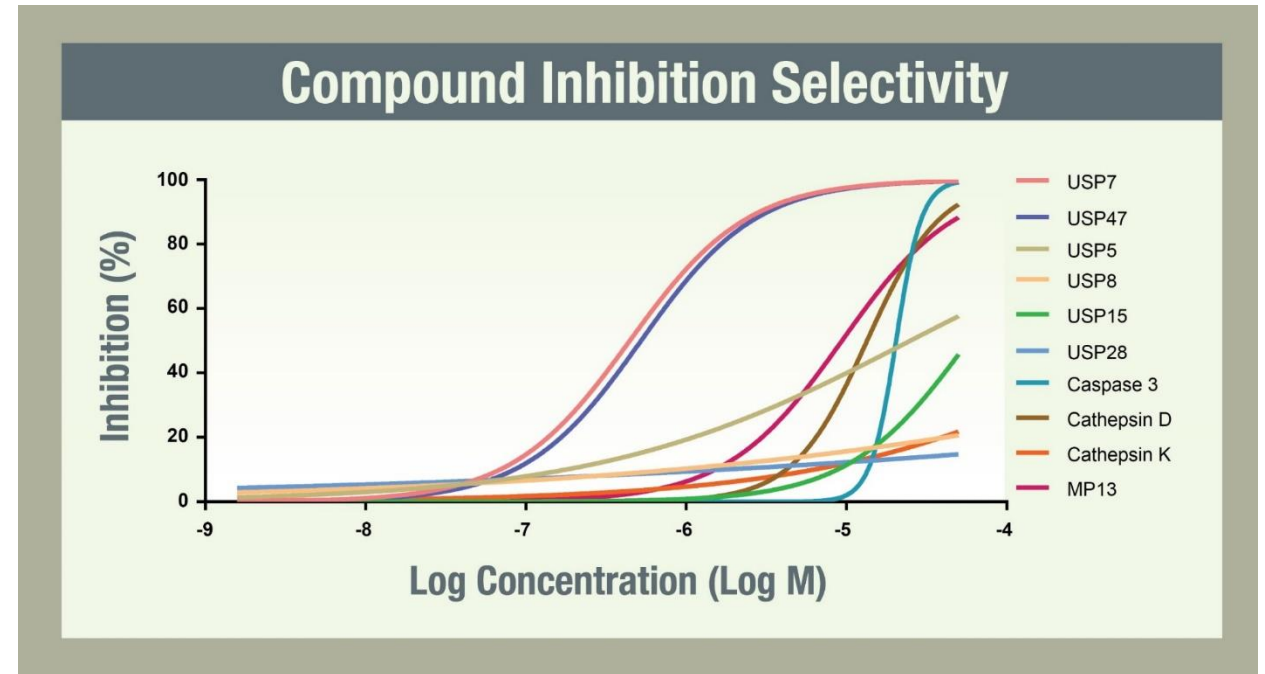
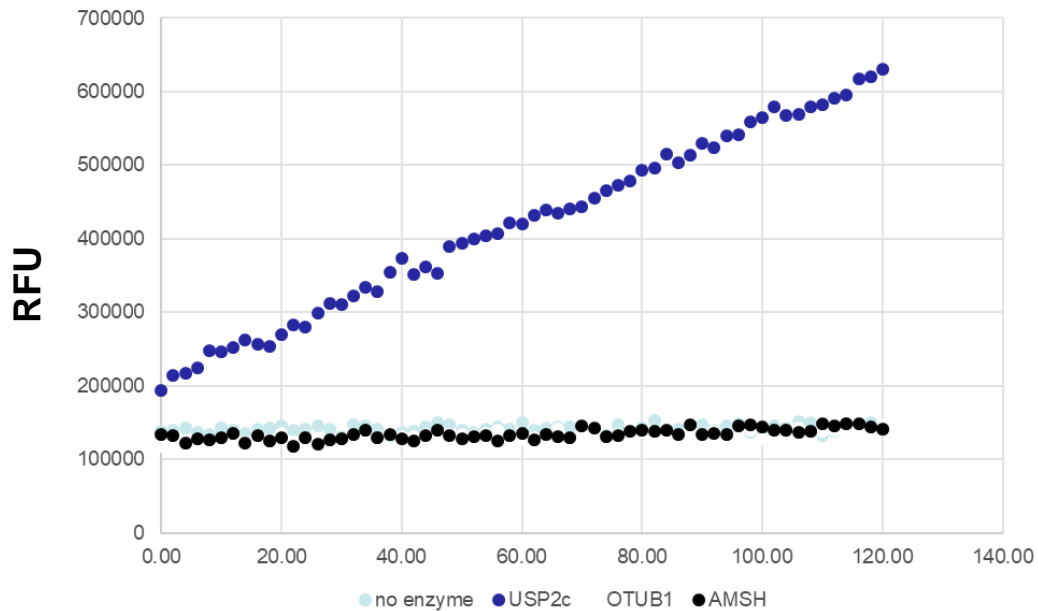


The **Ubiquitin Fluorophore Assay** utilizes a ubiquitin molecule bound to a quenched fluorophore (e.g. [Rhodamine 110](#)). After DUB digestion the unconjugated fluorophore is detected by measuring the change in fluorescence on a plate reader. This simple and straight-forward assay is a great high-throughput option for most DUBs.

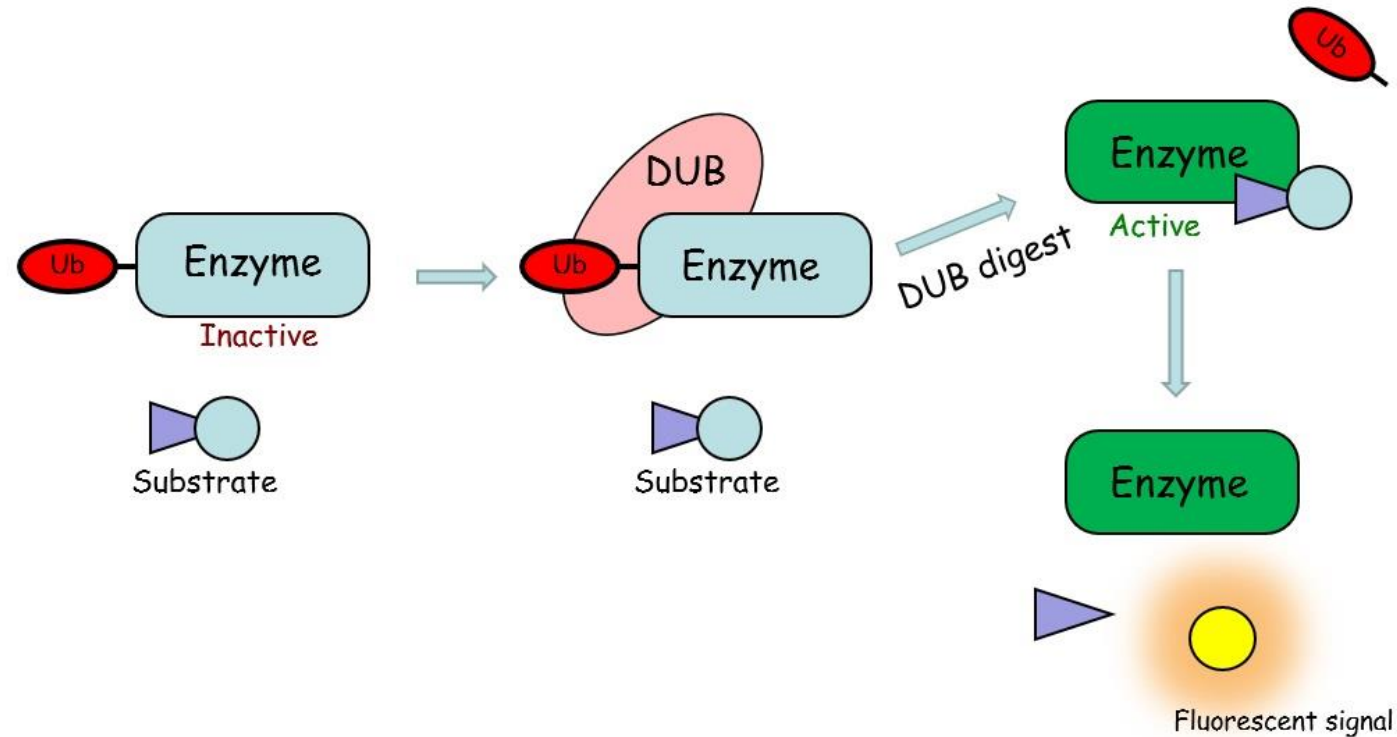
# Example of DUB HTS and Validation Assays

DUB activity with Ub-Rho 110 in the presence of a compound specific for USP7

DUB activity with Ub-Rhodamine 110



# DUB HTS and Validation Assays



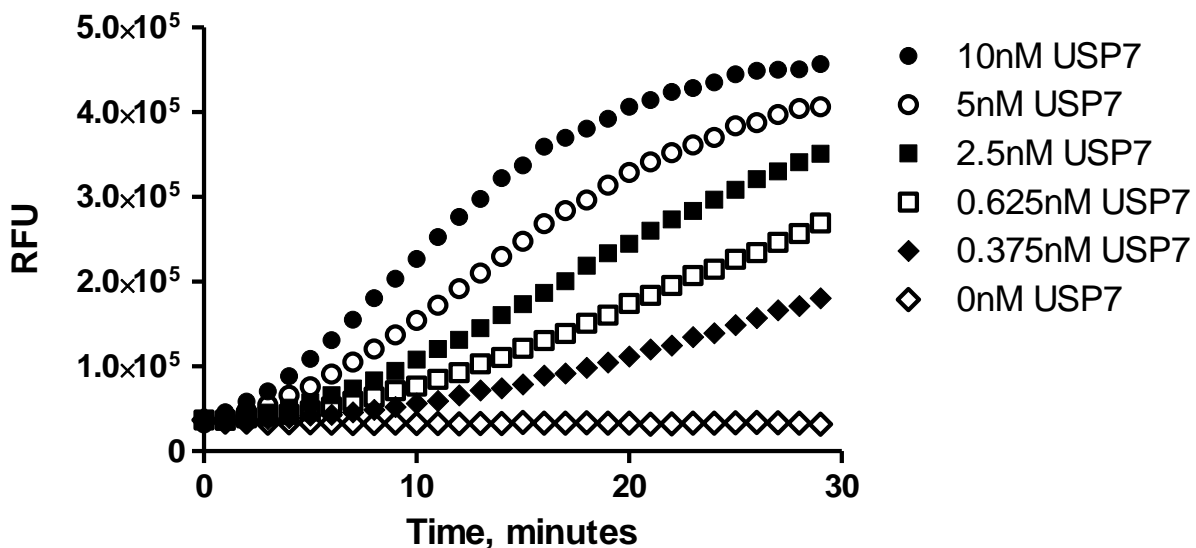
The **Ub-CHOP-Reporter Deubiquitylation Assay Kit** consists of ubiquitin fused to a reporter enzyme. Fusion of the N-terminus of the reporter to the C-terminus of ubiquitin renders the reporter catalytically inactive. Following cleavage of the Ub-reporter system by DUBs, the free (and now active) reporter enzyme acts on its fluorescence substrate. Thus, in this coupled assay, the signal generated by cleavage of the reporter enzyme's substrate is a quantitative measure of DUB activity.



# Example of DUB HTS and Validation Assays

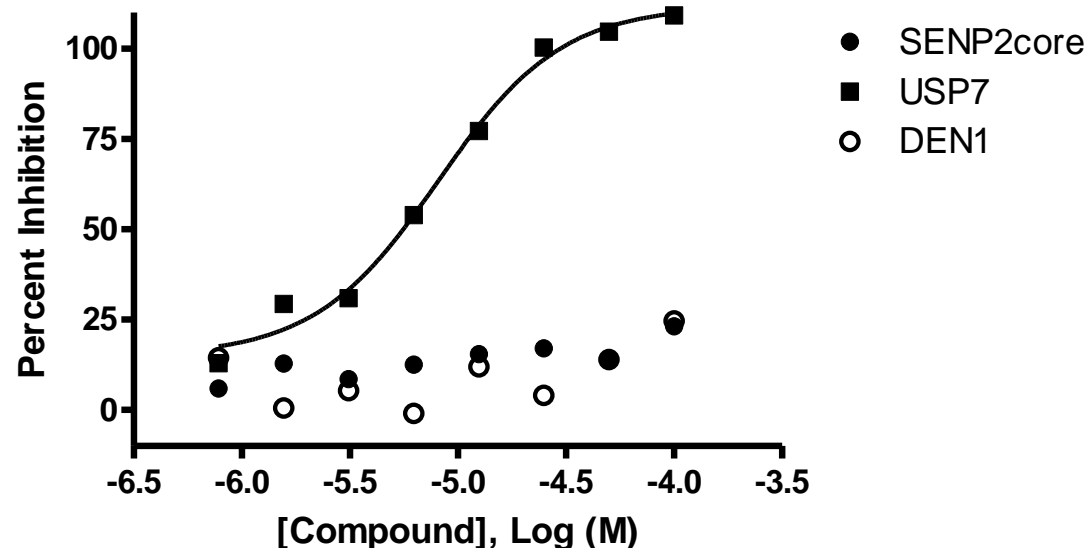
## CHOP Assay

### Dose response of USP7 in CHOP assay

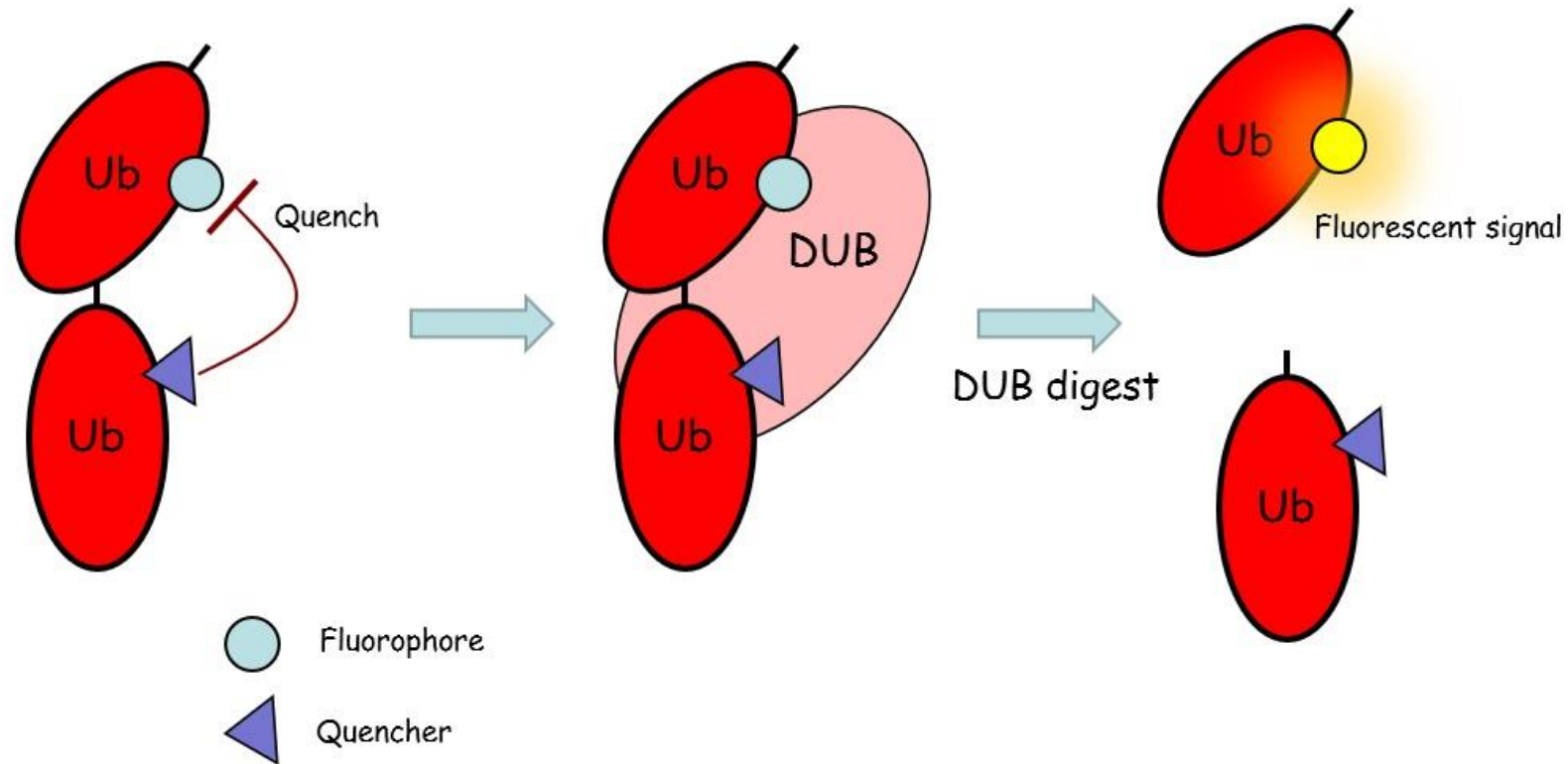


### IC50 values of USP7 inhibitor compound X

SENP2core and DEN1 are negative controls



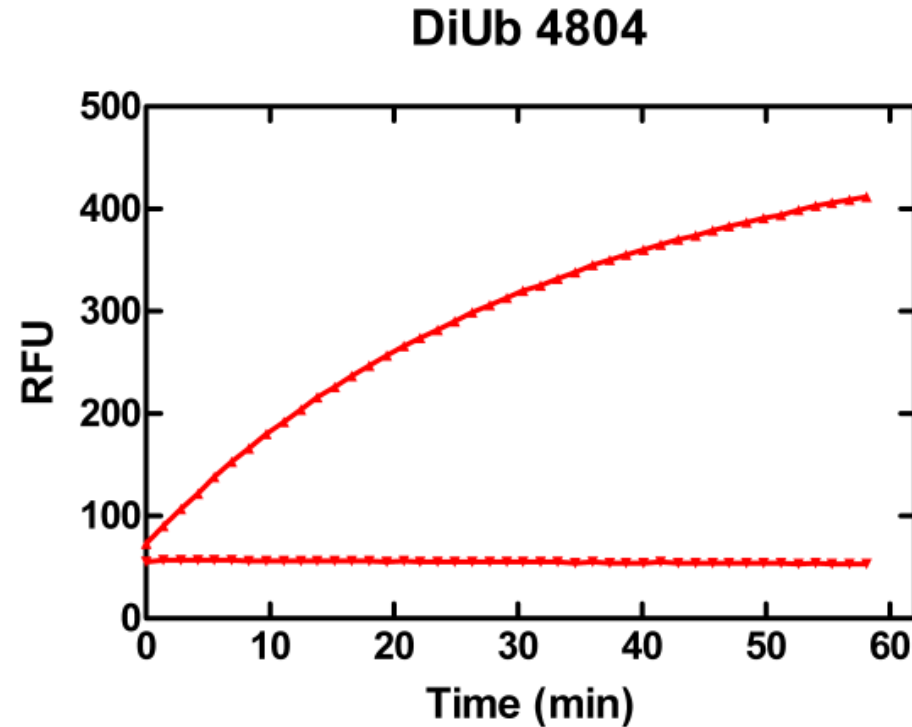
# DUB HTS and Validation Assays



The **IQF Assay** utilizes a pair of conjugated ubiquitin proteins. One carries a fluorophore silenced by the nearby quencher. After DUB digestion the free fluorophore can be detected by a plate reader. This assay is physiologically relevant since the DUB cleaves a Ub-Ub bond.

# Example of DUB HTS and Validation Assays

Internally quenched fluorescent FRET pair (IQF) assay



## Progression of DiUb cleavage by USP2core

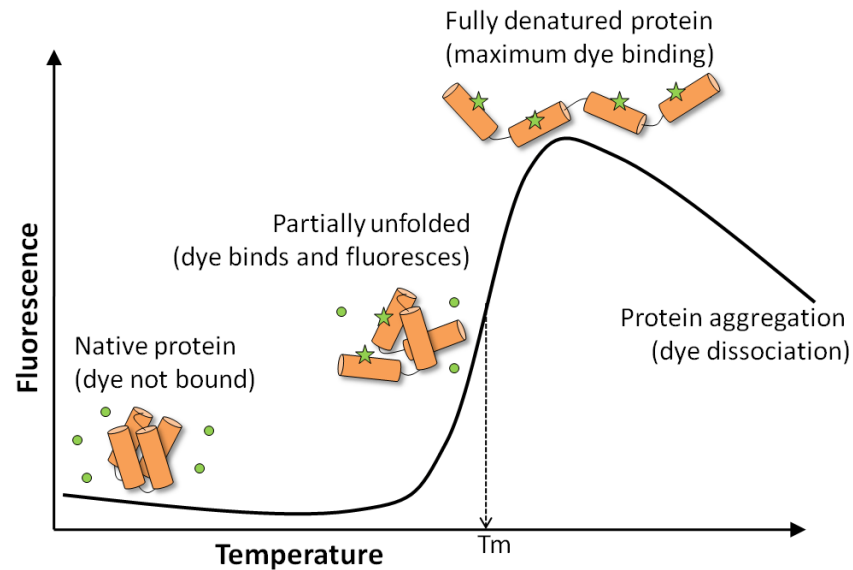
DiUb K48-4 (200 nM) was incubated with (closed symbols) or without (open symbols) 10nM USP2core. The Increase in TAMRA fluorescence was monitored.

# Step Two: Hit-to-lead optimization

## Validation Assays

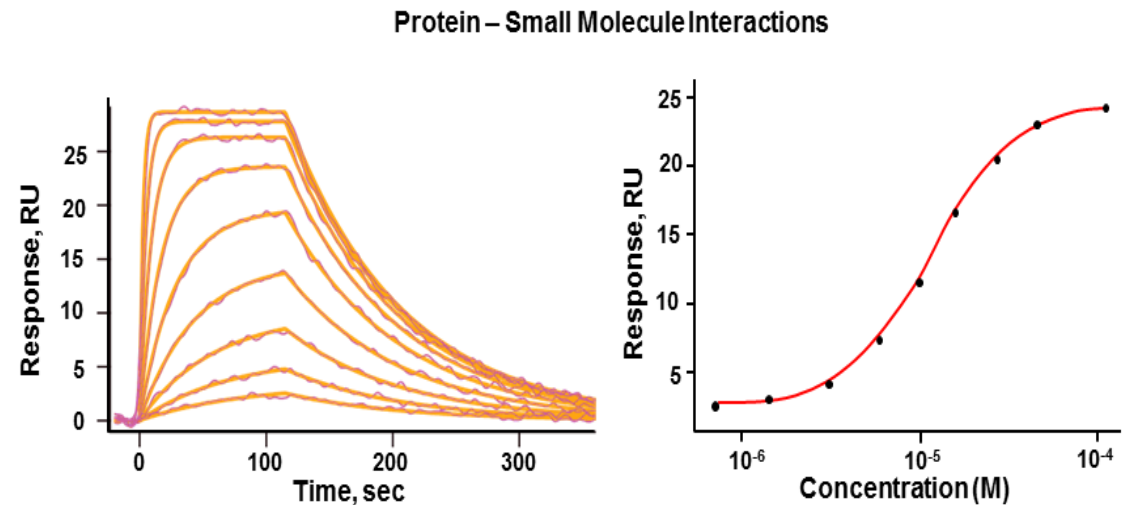
### Thermal Shift Assay

HTS assay to detect compound binding to a target

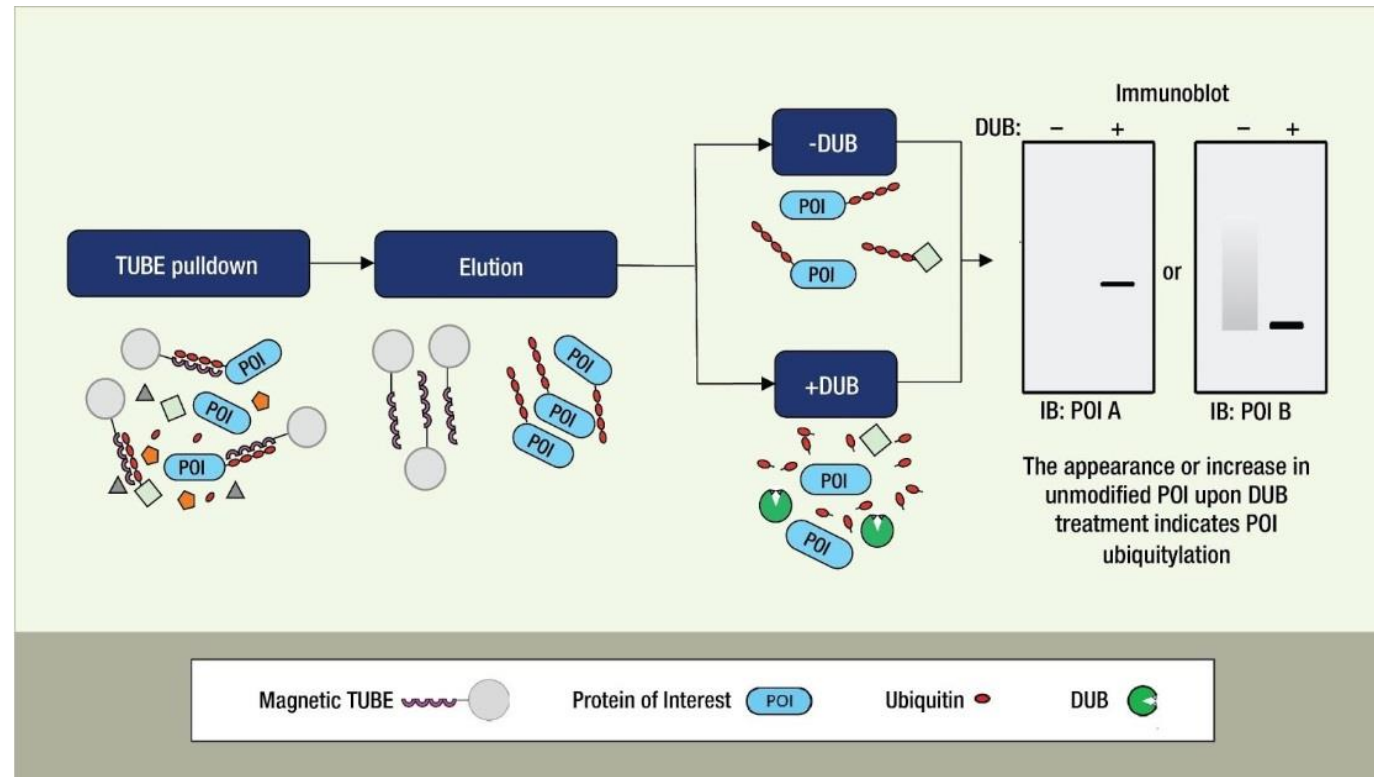


### Surface Plasma Resonance

Determination of a small molecule affinity to a target

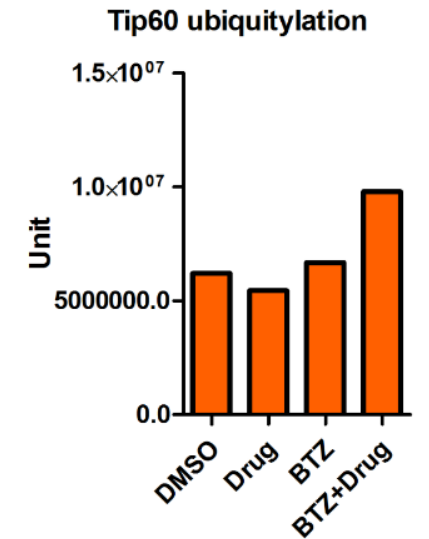
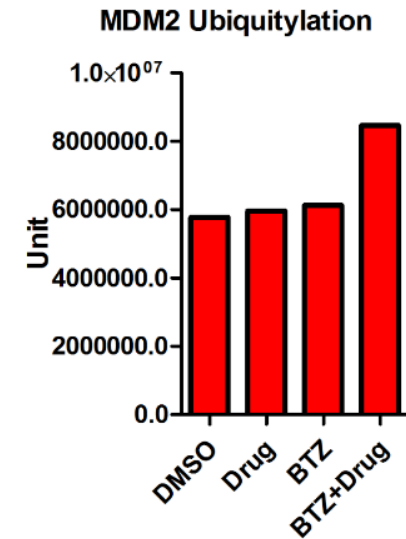
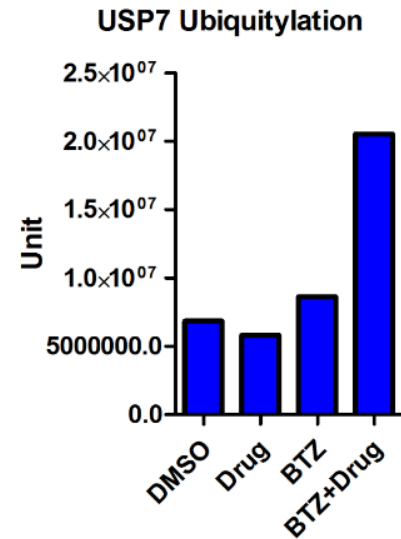
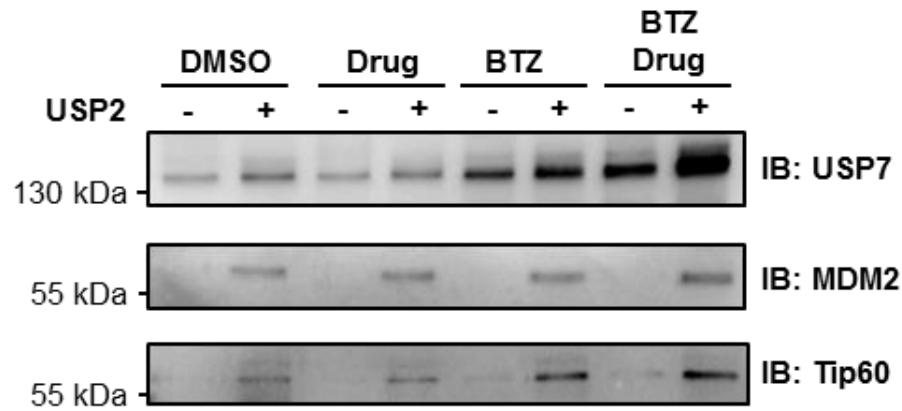


# UbiTest: Cellular Assay to Measure Ubiquitylation of POI



**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.

# LifeSensors DUB Selectivity Panel

(largest collection of functional DUBs in the industry and growing)

DUB Panel	Representative DUBs
<b>Panel I</b> (7 DUBs)	UCHL1, USP8c, USP15, USP7, USP4, USP2c, USP30.
<b>Panel II</b> (20 DUBs, includes DUB from panel I as well)	UCHL1, UCHL3, UCHL5, USP8c, USP15, USP7, USP4, USP2c, USP30, SENP1c, USP47, USP9x, USP21, USP10, OTUB1, YOD1, OTUB2, OTUD6B, Cezanne, A20.

Each DUB assay has been validated. LifeSensors profiles inhibitory or activation properties of every compound in Panel I followed by Panel II.

## DUB Screening & Profiling Service

- **Help customer discover DUB inhibitors or activators**
- **Express biologically active DUBs and substrates**
- **Develop and optimize HTS assay for DUB**
- **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 DUB drug discovery

## Contact Information

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