# Stabilizing Proteins; The Future of Breakthrough Drugs – DUBTACs

## DeUBiquitinating TArgeting Chimeras

# LifeSensors Inc. Mission

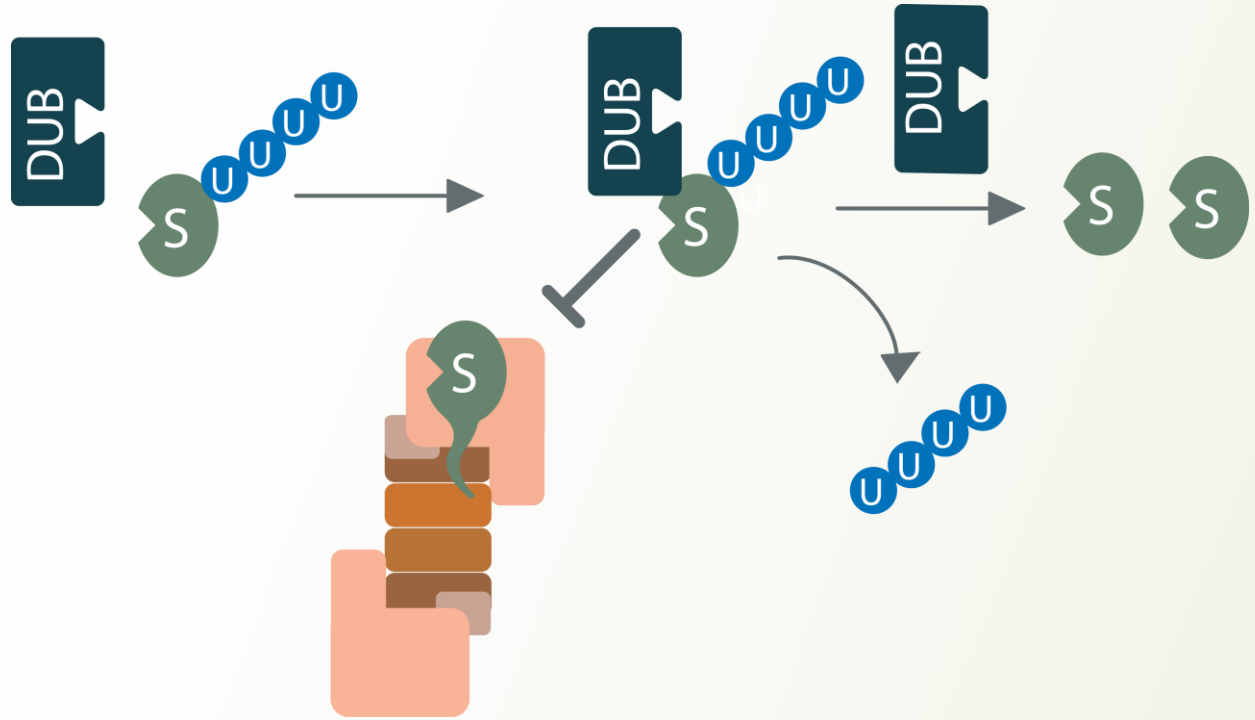
- Leadership in UPS, PROTAC, DUBTAC, and Molecular Glues
- Drug Discovery, UPS Enzymes, DUBs, PROTAC Screening Services
- Biomarker Development and Collaborative Research
- ~500 Products, DUBs, E3 ligases, Ubiquitin Affinity Matrices (TUBEs), Assay Kits and Proprietary Protein Expression Systems (SUMO)
- Profiling Compounds Against Ubiquitin Ligases and DUBs

# LifeSensors DUB Capabilities

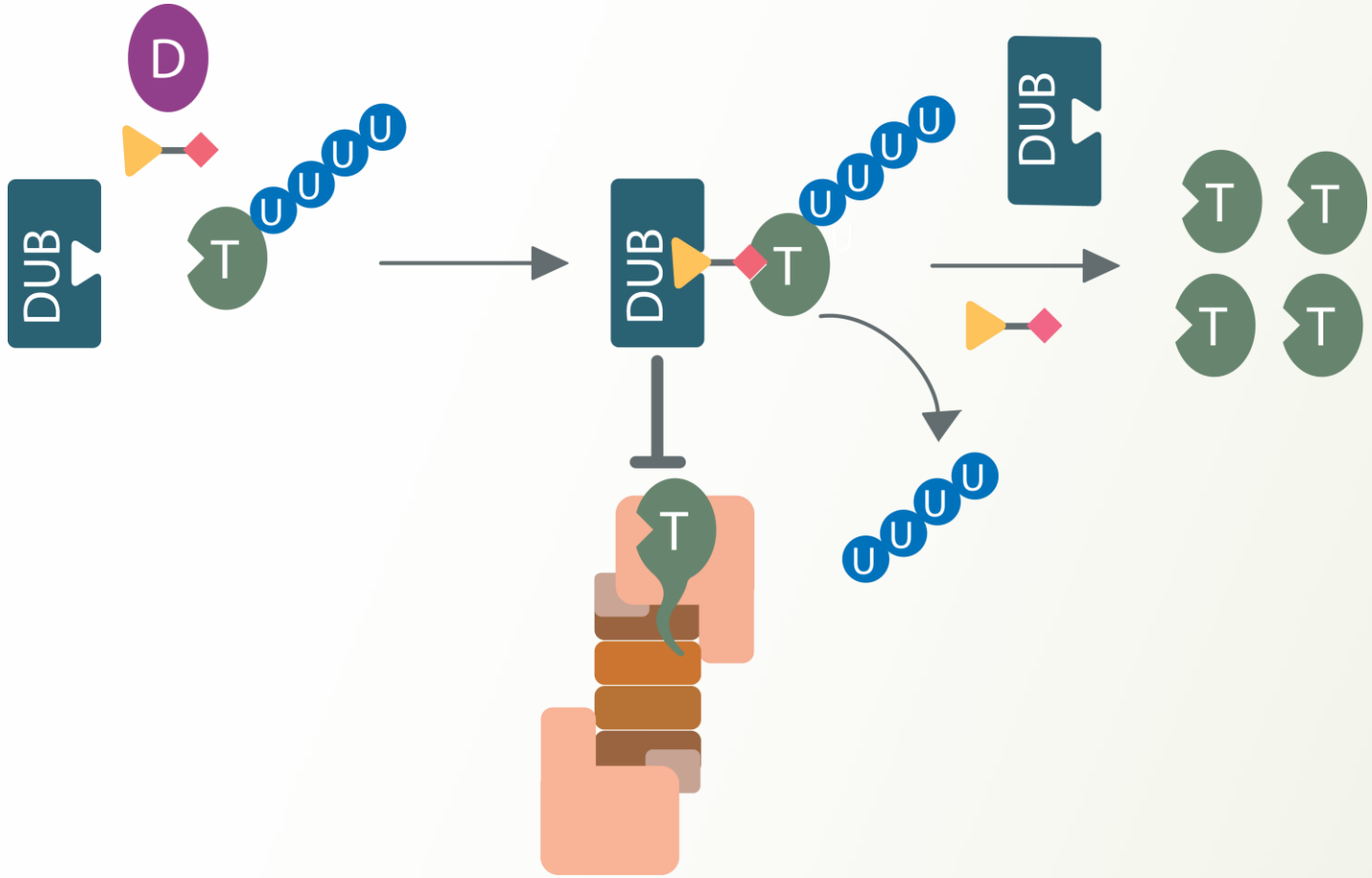
- Expressed/purified ~40 biologically active DUBs and substrates
- Developed ~25 physiological DUB assays for HTS and validation
- Selectivity panel and compound profiling
- Determine compound MOA, cellular and target tissue PD markers
- Ability to screen ~500,000 compounds

# DUB Functions

- DUBs are a large group of proteases that remove ubiquitin from proteins
- Rescue ubiquitylated proteins from proteasome attack
- Antagonize E3 ligase activity
- Regulate protein trafficking, autophagy
- Epigenetic chromatin remodeling; DUBs are “erasers”



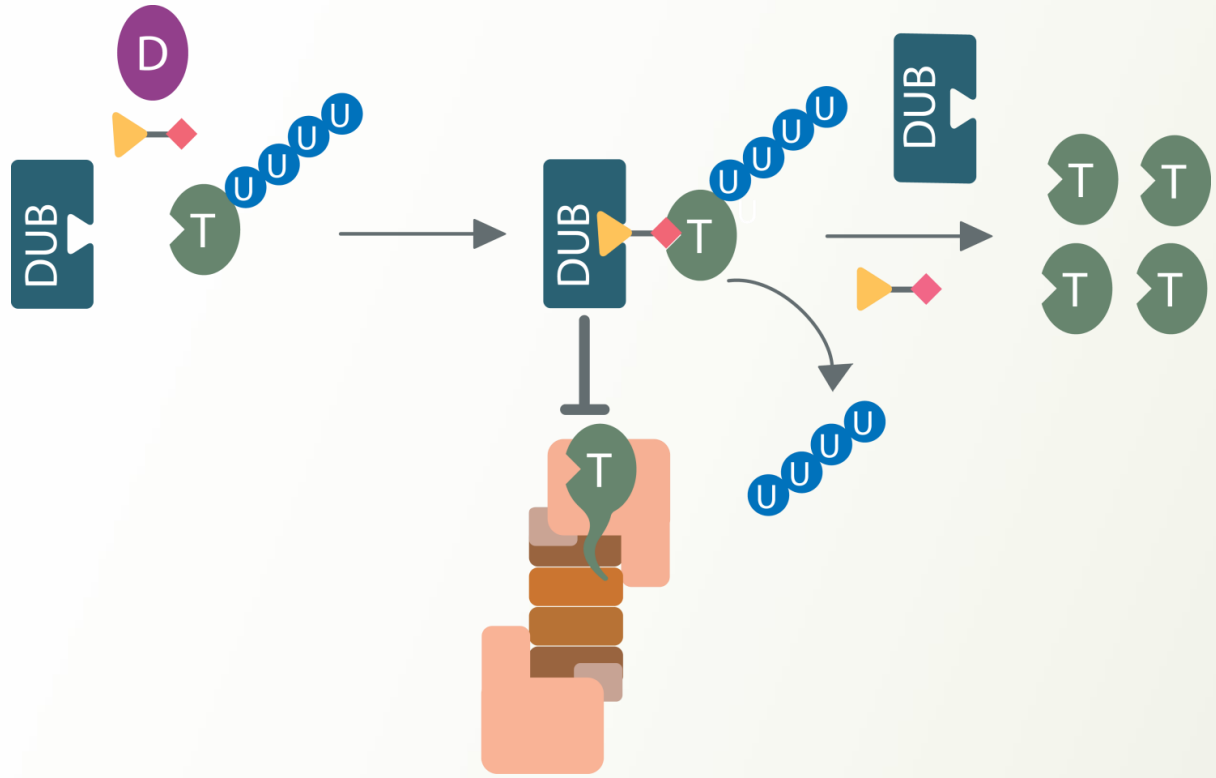
# DeUBiqutinating TArgeting Chimeras: DUBTACs



# DUBTAC: An Emerging Therapeutic

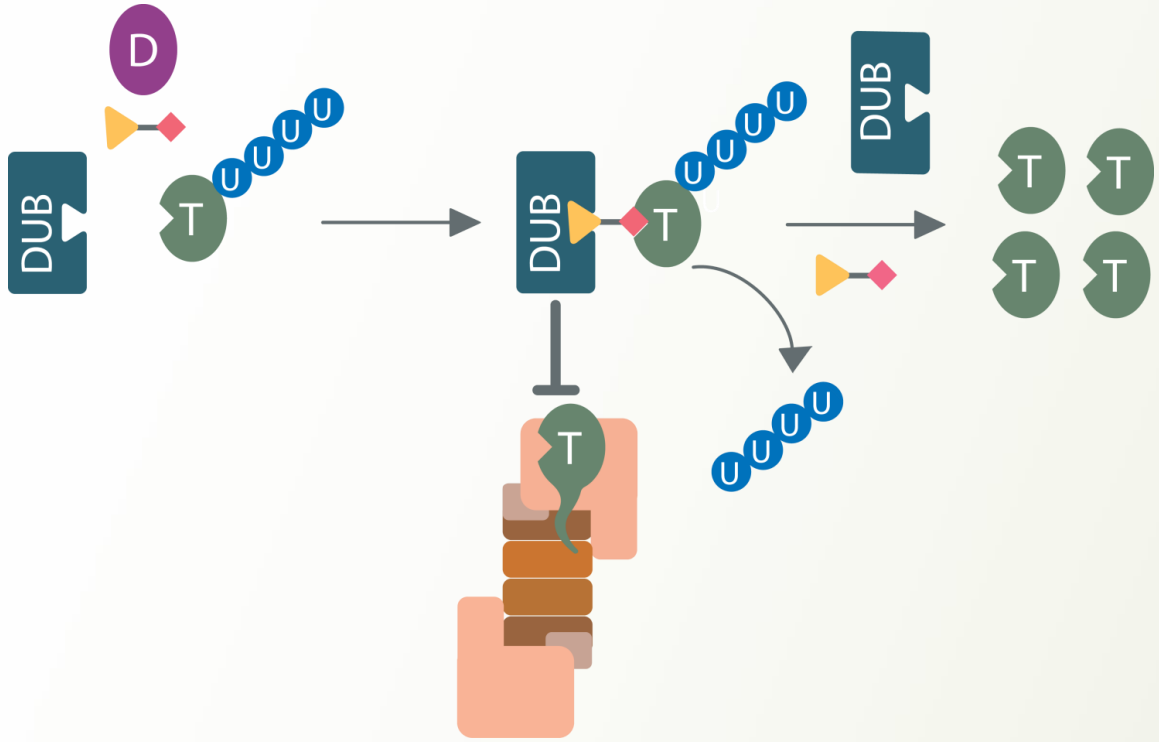
- DUBTACs recruit DUBs to a target protein and remove ubiquitin chains, resulting in stabilization of target proteins

- DUBTACs consist of three components:
  - DUB recruiter
  - Target protein binder
  - Linker connecting both entities



# Harnessing DUBs for Targeted Protein Stabilization

- Traditional PROTACs promote *degradation* of target proteins, whereas DUBTACs *stabilize* target proteins
- DUBTACs restore protein levels, function, and rescue target proteins from degradation via the proteasome



# LifeSensors' Approach to DUBTAC Drug Discovery

1

**Validation of DUBTAC Ligands for Stabilizing Activity rather than Inhibition**

- Ub-Rh110/Ub-AMC assay
- CHOP reporter assay
- Diubiquitin internally quenched fluorescence (IQF) assay
- UbiSensor assay (TR-FRET)[New****]

2

**Cellular Validation & DUBTAC Activity**

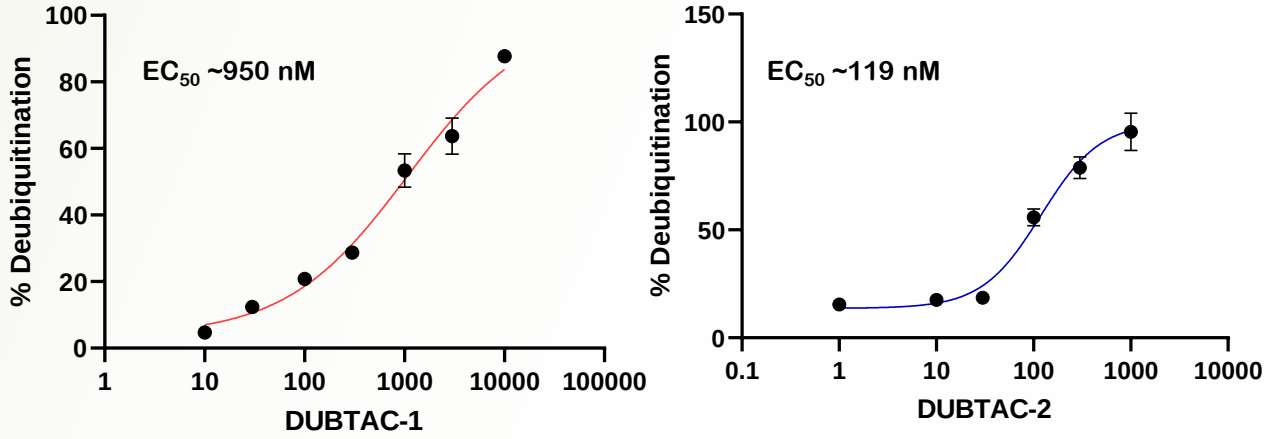
- UbiTest Assay
- UbiQuant ELISA

3

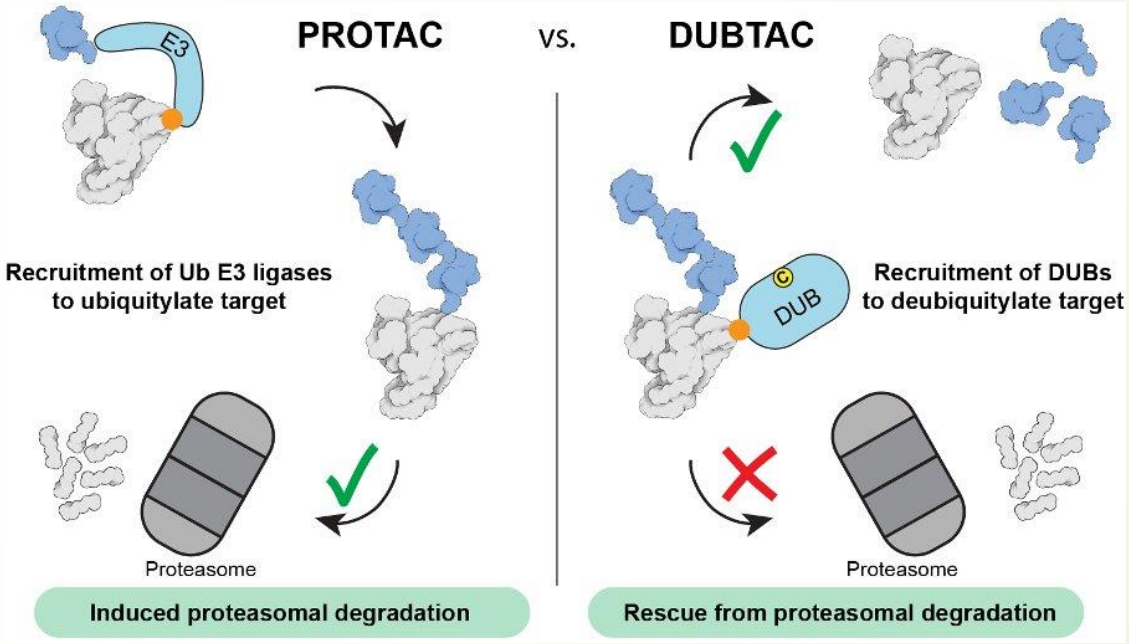
**Specificity Studies**

- TUBE-based mass-spectrometry studies

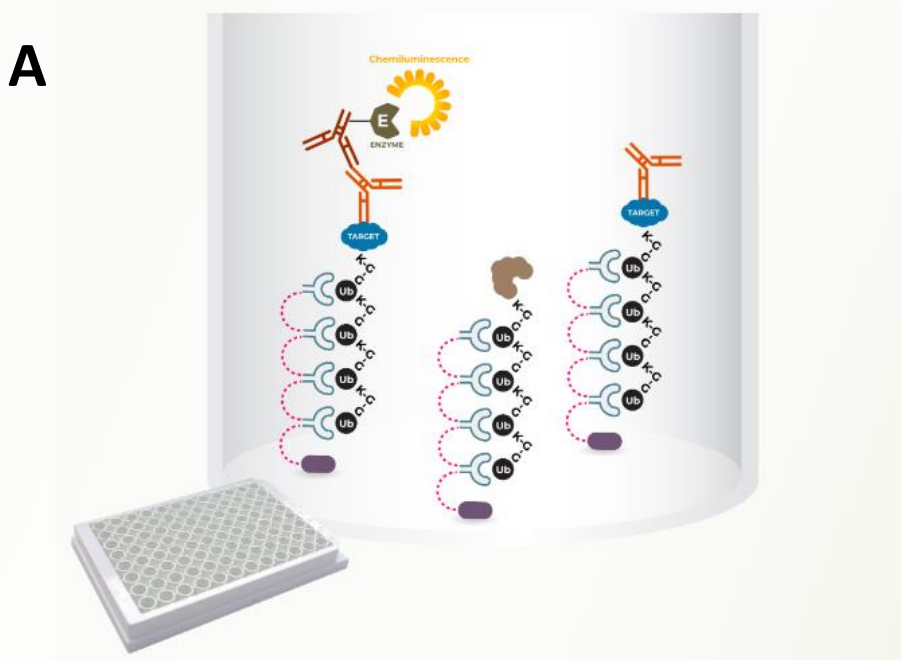
# Rapid EC50 determination of DUBTACs using Lifesensors' assay platform

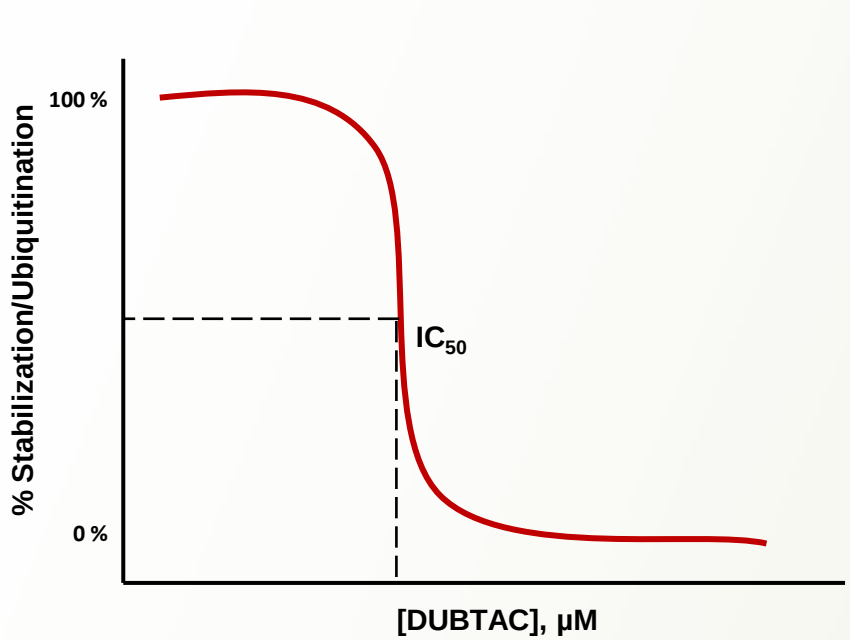



Monitoring deubiquitination – changes in compound mediated ubiquitination profiles mediated by DUBTAC to establish quantitative ubiquitination signals along with demonstrating $EC_{50}$'s of two test compounds



# UbiQuant ELISA for Cellular Validation of DUBTAC Activity




**(A)** UbiQuant ELISA to study ubiquitination and subsequent de-ubiquitination in presence of DUBTAC that represents stabilization of protein of interest. UbiQuant ELISA uses TUBE technology to estimate and accurately quantitate ubiquitinated proteins of interest with sub-nanomolar affinity. **(B)** A dose response study with DUBTAC suggests loss in basal ubiquitination on target protein with increase in DUBTAC dose representing successful protein stabilization.

# A New Perspective to the Ubiquitin Proteasome System

Adaptation of the Ubiquitin Proteasome System (UPS) presents an abundant space for drug discovery

DUBs have been implicated in many regulatory mechanisms and biological functions, where they play a role in a plethora of clinical disease

LifeSensors helps you study these DUBs to make breakthrough discoveries and pave the way to novel therapeutics

# DUB Screening & Profiling Services

- Discover novel DUB ligands by HTS assays
- Confirmation and counter screen to eliminate off-target compounds
- Biophysical and biochemical assay development for DUBs
- Cell-based assays to establish target engagement by compounds
- Fee for service model, defined milestone-based agreement

# Thank You

**We are your partner in UPS, DUBs, E3s, PROTAC, Mol Glue, Protein Expression, CAR-T/Gene therapy and vaccine development**

# Contact Us!


| | | | |
|---|---|---|---|
| **Research & Product Inquiries** | **R&D** | **info@lifesensors.com** | **610-644-8845 (ext 339)** |
| **Custom Service & Assays** | **BD** | **bd@lifesensors.com** | **610-644-8845 (ext 310)** |