# LifeSensors DUBs Screening & Profiling Platform

### LifeSensors

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# LifeSensors Inc. Mission

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

# **Ubiquitin Proteasome System**



 I - Ubiquitin activating enzyme Requires ATP to attach Ub to E1
Ubiquitin conjugating enzyme Transfers Ub from E1 to E3
Ubiquitin ligases Transfers Ub to self or substrate Forms mono-Ub or poly-Ub chains
Dubiquitinase Removes mono-Ub or poly-Ub chains
Degrades ubiquitylated proteins

PROTEASOME

# **DUB-based Drug Discovery Capabilities**

- ~40 biologically active DUBs for compound profiling and selectivity panel
- Developed <u>~25 DUB assays</u> for a variety of HTS and validation strategies
- Ability to screen ~500,000 compounds
- Establish pre-clinical and clinical biomarkers for DUB, DUBTAC ligands
- Determine compound MOA, cellular and target tissue PD markers

# **DUB Families**

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

# **LifeSensors DUB Selectivity Panel**

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

DUB Panel	Representative DUBs
Panel I (10 DUBs)	USP2c, USP4, USP5, USP7, USP8c, USP15, USP30, UCHL1, OTUB1, OTUD7B (Cezanne).
Panel II (22 DUBs, includes DUB from panel I as well)	USP2c, USP4, USP5, USP7, USP8c, USP10, USP15, USP21, USP28, USP30, USP33c, USP47, OTUB1, OTUB2, AMSHc, Cezanne, UCHL1, UCHL3, UCHL5, SENP1c, SENP6c, PLPro.

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

# **Step One: DUB Assay Development, Optimization and HTS**

#### **DUB HTS and Validation Assays**



- The classic <u>Ubiquitin Fluorophore Assay</u> utilizes a ubiquitin molecule attached to a quenched fluorophore (e.g. Rhodamine 110).
- DUB activity releases the fluorophore resulting increased fluorescence.
- This simple and straight-forward assay is a great highthroughput screening option for most DUBs.

# **Example of DUB HTS and Validation Assays**



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# **DUB HTS and Validation Assays**

**Ub-CHOP-Reporter Deubiquitylation Assay** 



- The <u>Ub-CHOP-Reporter Deubiquitylation Assay Kit</u> consists of ubiquitin fused to a reporter enzyme.
- The fused reporter enzyme stays catalytically inactive.
- Cleavage of the ubiquitin by DUBs, releases the active reporter enzyme which acts on its fluorescence substrate.
- The fluorescence signal generated by the reporter enzyme 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



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# **DUB HTS and Validation Assays**

Internally Quenched Fluorescently Labeled DiUbiquitin assay



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

# **Example of DUB HTS and Validation Assays**

Internally Quenched Fluorescently Labeled DiUbiquitin assay



### DiUb 4804

- Progression of DiUb cleavage by USP2core
- DiUb K4804 (200 nM) was incubated with (curve) or without (flat line) 10nM USP2core.
- The increase in fluorescence was monitored.

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# A novel TR-FRET DUB assay platform (UbiProbe) (Highly <u>sensitive</u>, <u>robust</u> and <u>HTS compatible</u> DiUb assay)



#### **Key Features**

- Physiologically relevant DiUb substrates with native isopeptide linkages
- Highly sensitive (lower enzyme dose) and robust assay
- Far red shifted fluorophores ideal for HTS and compound screens
- Suitable for discovering/profiling DUB ligands, inhibitors and activators
- Better substitute for IQF DiUbiquitins

### **UbiProbe Assay Applications: DUB inhibitor profiling**



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#### PR-619

- PR-619 is a widely used pan DUB inhibitor
- PR-619 IC50 against USP7 and USP21 was determined using K48-DiUb in UbiProbe assay
- PR-619 inhibits USP7 with an IC50 of 0.66 μM
- PR-619 inhibits USP21 with an IC50 of 1.01 μM

### **UbiProbe Assay Applications: HTS Assay Validation**

(DUB with K63 DiUb)

HTS Assay Z'



- UbiProbe assay was validated for HTS using a K63-chain specific DUB
- ~13K compounds (40 plates) screened in 384-well format
- Z' >0.6 across the 40 plates

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- S/B =3.5
- CV= 7.44%



- USP2 is a pan Ubiquitin chain DUB
- USP2 DUB is active in Di-Ub linkages tested



#### **OTUB1** K48-Specific DUB

- OTUB1 is a K48-Ubiquitin Chain specific DUB
- OTUB1 activity is only observed with K48-DiUb



- AMSH is a K63-Ubiquitin Chain specific DUB
- AMSH activity is only observed with K63-DiUb

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#### OTUD7B/Cezanne K11-Specific DUB

- OTUD7B/Cezanne is a K11-Ubiquitin Chain specific DUB
- OTUDB7B/Cezanne is only active with K11-DiUb

# **UbiProbe Assay SUMMARY**

- UbiProbe TR-FRET DUB assay:
  - Utilizes physiologically relevant DiUb substrates
  - Highly efficient and robust assay for HTS applications
  - Ideal for rapid compound profiling against DUBs
  - Suitable for studying DUB chain selectivity and mechanism of action
  - A better substitute for IQF DiUbiquitins

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

#### **Validation Assays**

#### **Thermal Shift Assay**

HTS assay to measure compound binding/affinity to the target DUB



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#### (SPR) Surface Plasma Resonance

Determines compound binding affinity to a target DUB



# **DUBTAC: An Emerging Therapeutic**

UB

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

DUB

- DUBTACs consist of three components:
  - DUB recruiter
  - Target protein binder

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• Linker connecting both entities

- 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



# 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

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# **UbiTest: Cellular Assay to Measure Ubiquitylation of POI**



#### <u>UbiTest</u> –

- A TUBE-based pull-down method that isolates total cellular ubiquitylated proteins.
- Subsequently, samples are treated with panselective 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**

Drug









#### Determine endogenous target protein ubiquitylation using UbiTest

- Jurkat cells were treated with indicated compounds and lysed in RIPA buffer. ۲
- Polyubiquitylated proteins were pulled down using TUBE1 agarose resin.
- Eluted proteins were incubated with DUB.

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Immunoblot (left) of the assay and quantitation (right) of the bands showed increased signal of USP7, MDM2, Tip60 after DUB treatment indicating they are polyubiquitylated in cells.

# **Determining** *In Vivo* Drug Activity by Cellular Biomarker Assays

### • UbiQuant S – ELISA

Medium-throughput relative quantification of substrate ubiquitylation levels in a cellular context.



# **DUB Screening & Profiling Service**

- 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



# **DUB Screening & Profiling Service**

#### **Pathway to DUB Modulator Drug Discovery**





# We are your partner for DUB drug discovery

### **Contact Information**

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