Role of Ternary Complex Formation and Ubiquitylation Assays in Early Protein Degrader Discovery

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Nurix Drugs Engage Ligases for the Treatment of Cancer

Targeted Protein Modulation: TPM = TPD + TPE

A Powerful Cellular System

Targeted Protein Elevation (TPE)

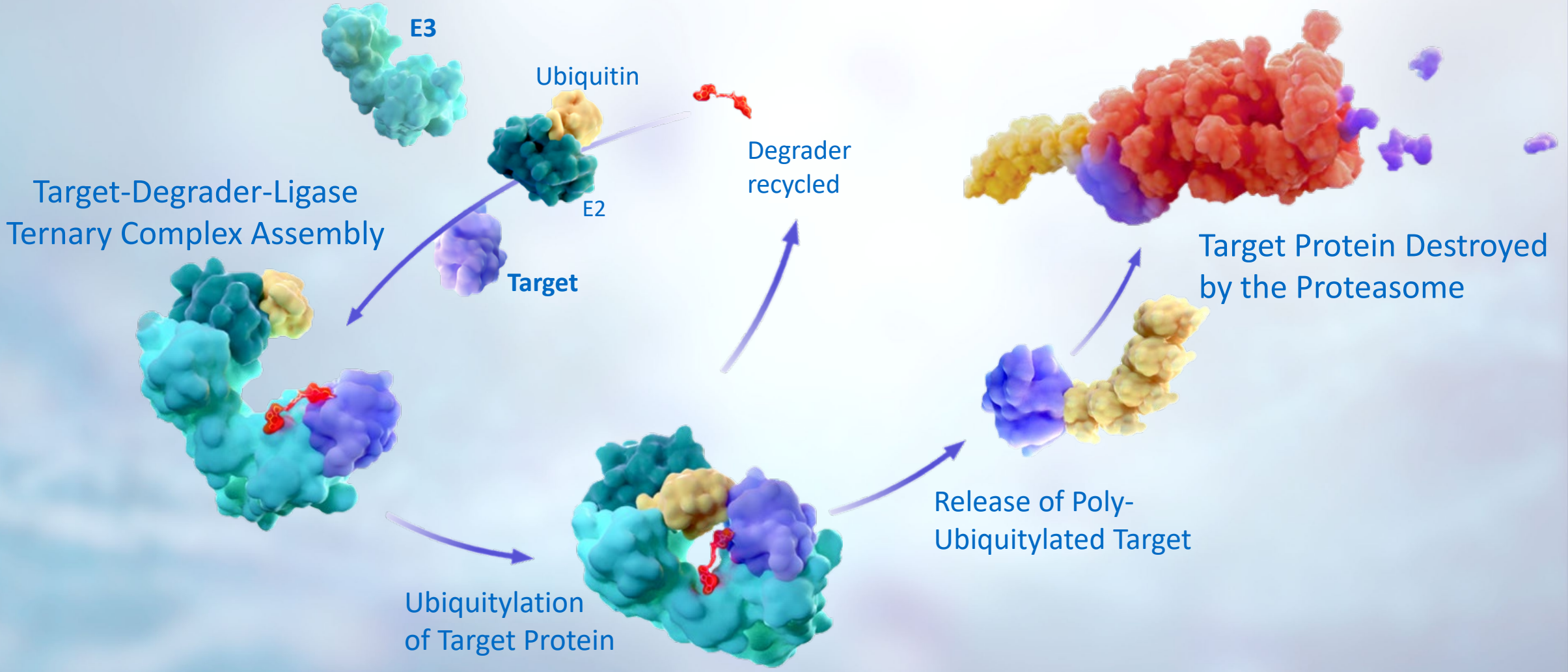
Inhibit ligases to increase specific protein levels

Harness ligases to decrease specific protein levels

Targeted Protein Degradation (TPD)

Ubiquitin is ligated to target proteins to tag them for degradation by the proteasome

Harnessing the Ubiquitin Proteasome System for Therapeutic Benefit



nurix

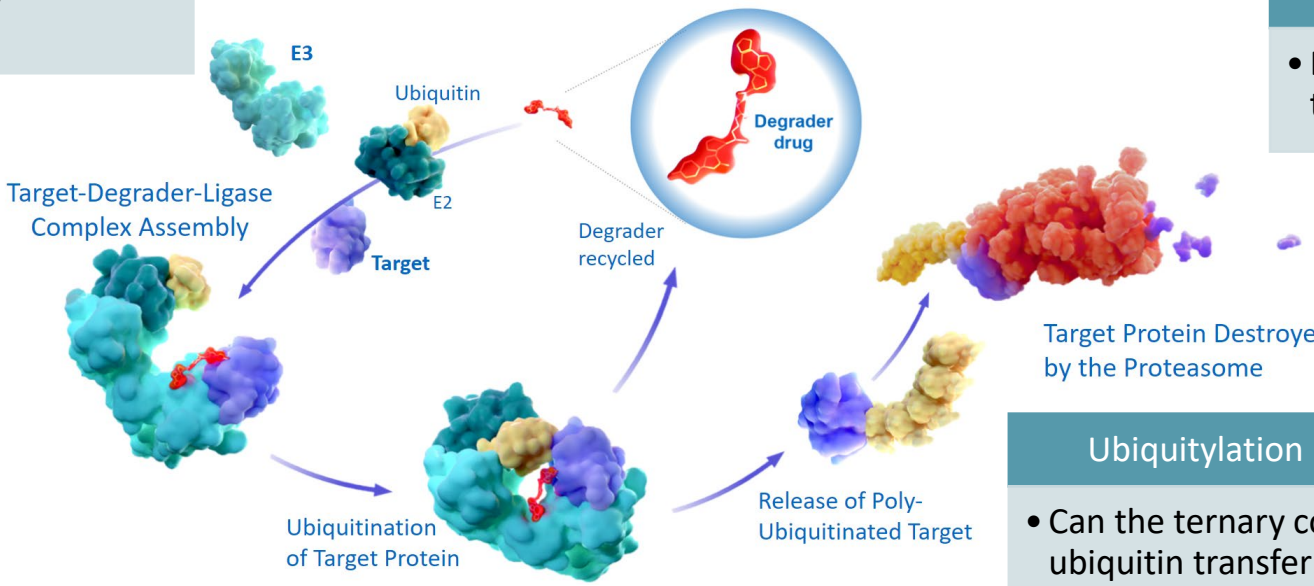
Assay Toolbox for Degrader Characterization

Probe displacement (TR-FRET)

- Does the degrader still bind the target and E3 with the same affinity as the original binders?

Ternary Complex (TR-FRET)

- Does the degrader engage target protein and ligase simultaneously?



Protein Degradation (HiBit Luminescence)

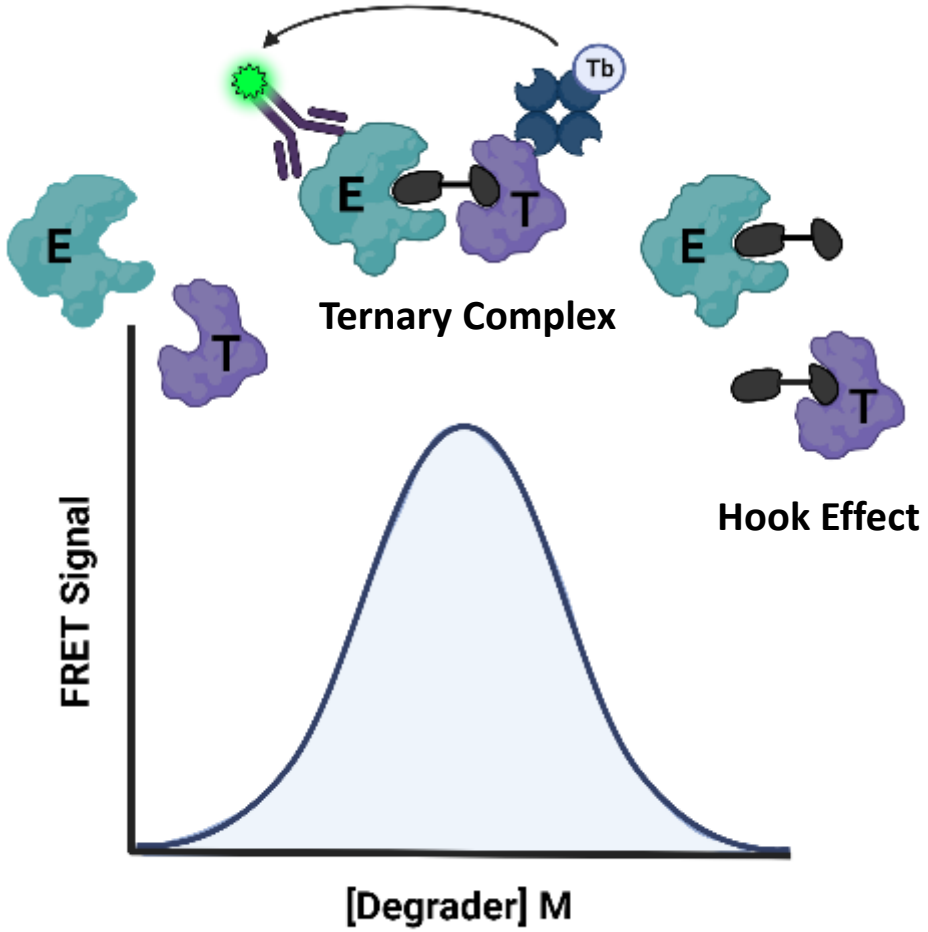
- Does the degrader eliminate the target protein in cells?

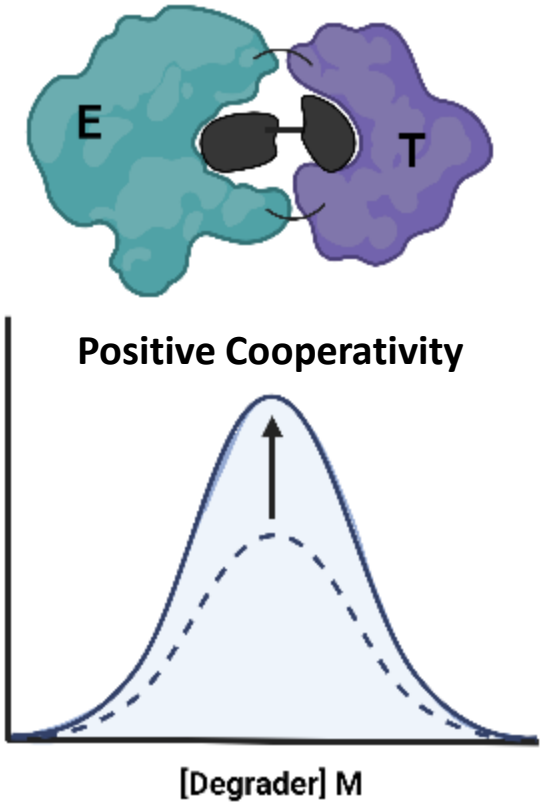
Ubiquitylation Activity (TR-FRET)

- Can the ternary complex enable ubiquitin transfer to target protein?

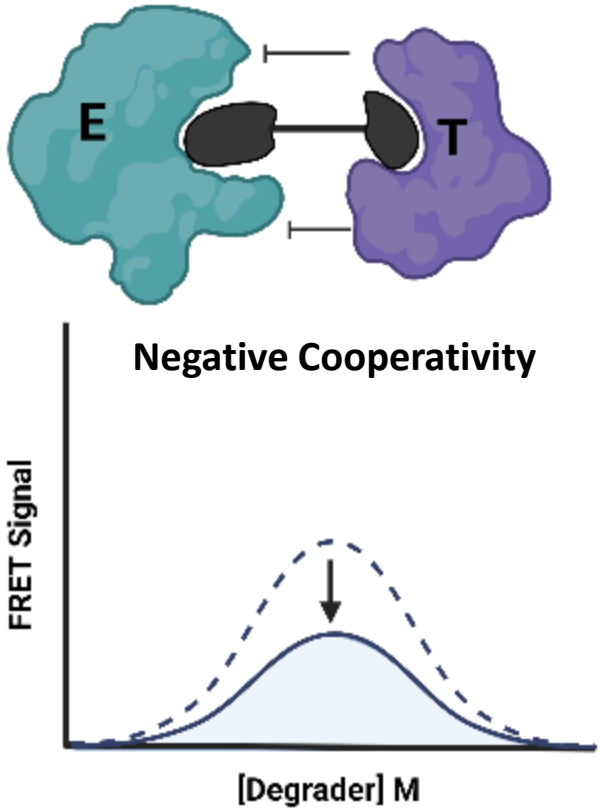
- These tools can be used to evaluate and optimize each step in the process
- These assay technologies can be scaled to rapidly profile degrader libraries

Ternary Complex Assay and Impact of Cooperativity





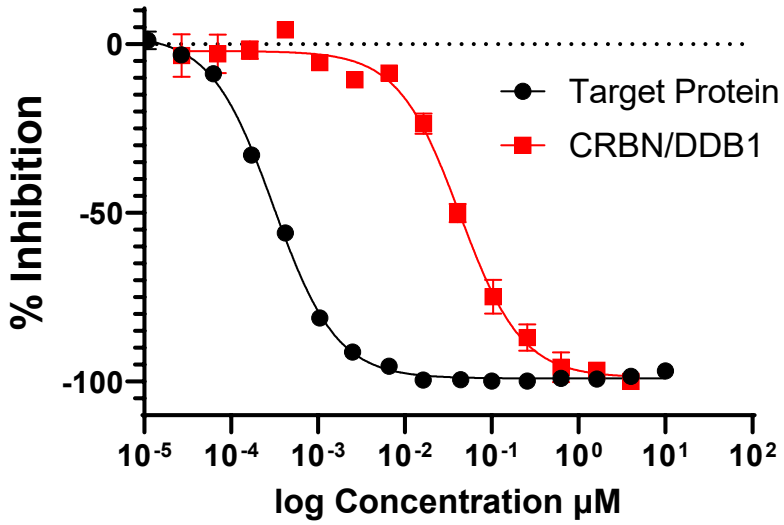
- Novel Protein-Protein Interactions
- Better resistance tolerance



- Antagonistic Protein-Protein Interactions
- Less stable ternary complex

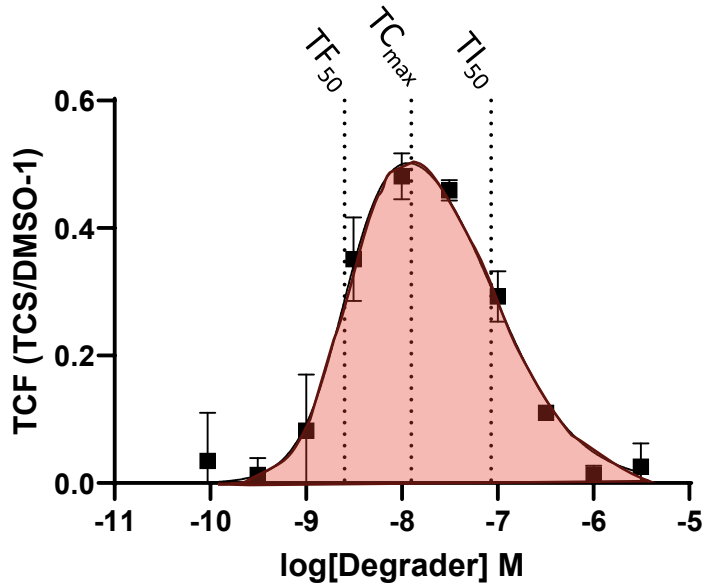
Parameters that Describe the Ternary Complex Bell Curve

Binary Binding
Standard Hill Equation



Fit Parameters	
Target Protein **IC_{50}**	0.3 nM
CRBN/DDB1 **IC_{50}**	43 nM

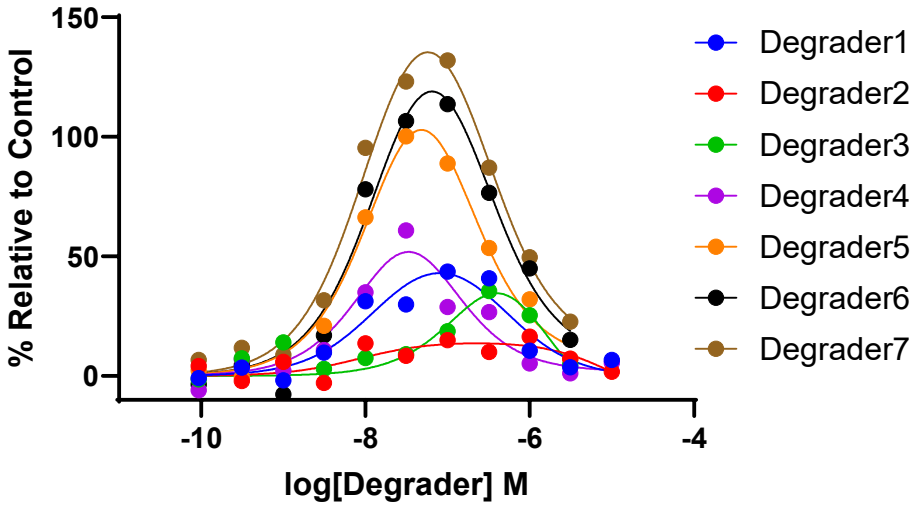
Ternary Complex
Biphasic Hill Equation



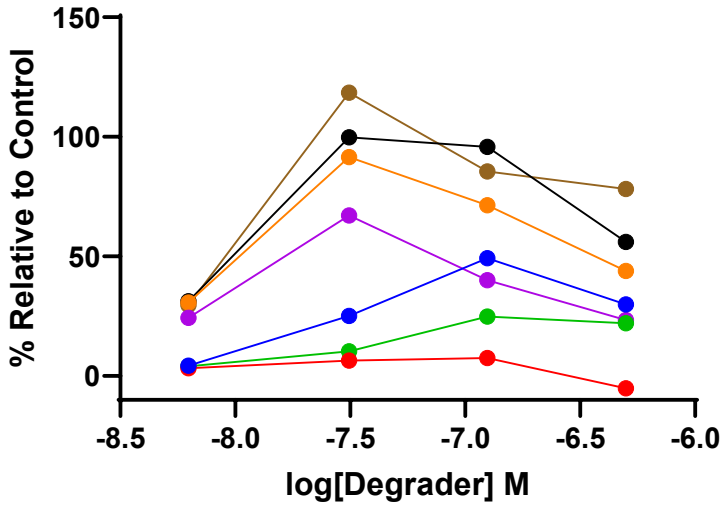
Fit Parameters		
TF_{50}	2.5 nM	½ maximum formation of ternary complex
TC_{max}	11 nM	Concentration of max ternary complex
TI_{50}	85 nM	½ maximum inhibition of ternary complex
AUC	0.93	Proportional to cooperativity (red shading)
%TC	0.5	Maximum TC signal observed

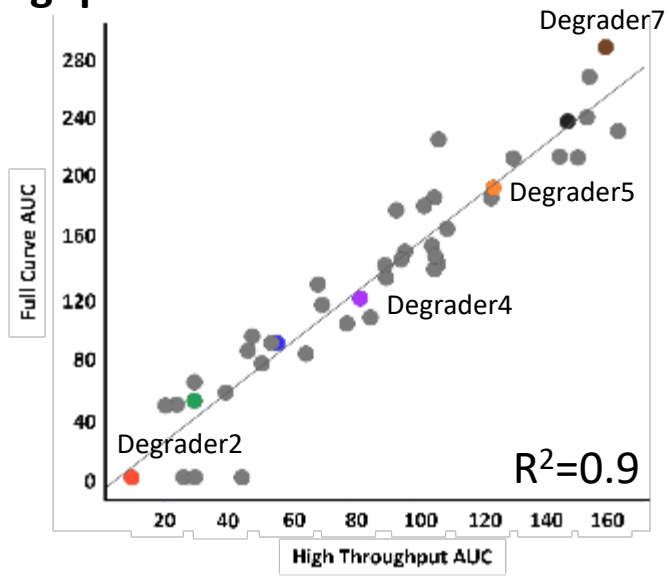
Ternary Complex Formation Assay is Scalable to High-Throughput

11-pt Dose Response



4-pt High Throughput Screen





Full Curve Assay

Determine TF_{50}, TI_{50}, TCmax, AUC, %TC

High Throughput Assay

Estimate AUC, %TC, TC_{max}

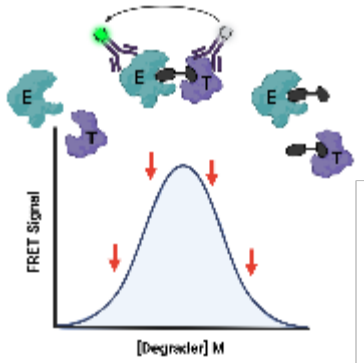
High Throughput AUC Captures Rank Order

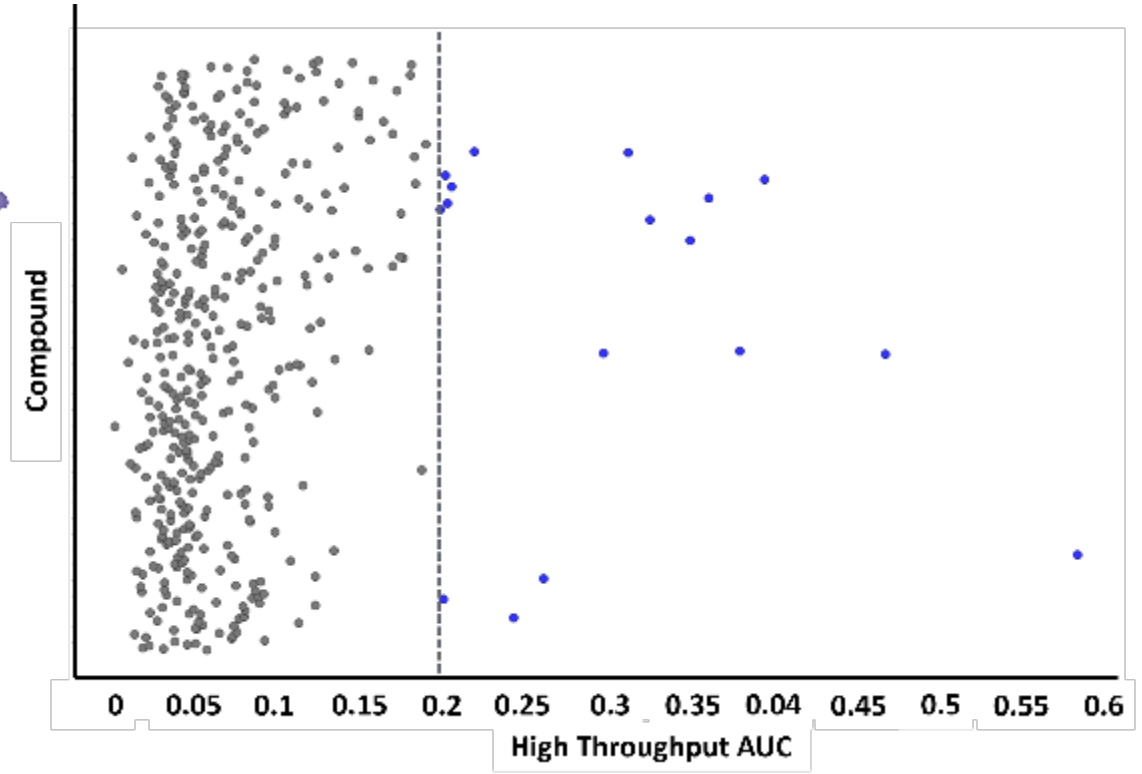
Advantages of 4-point screen:

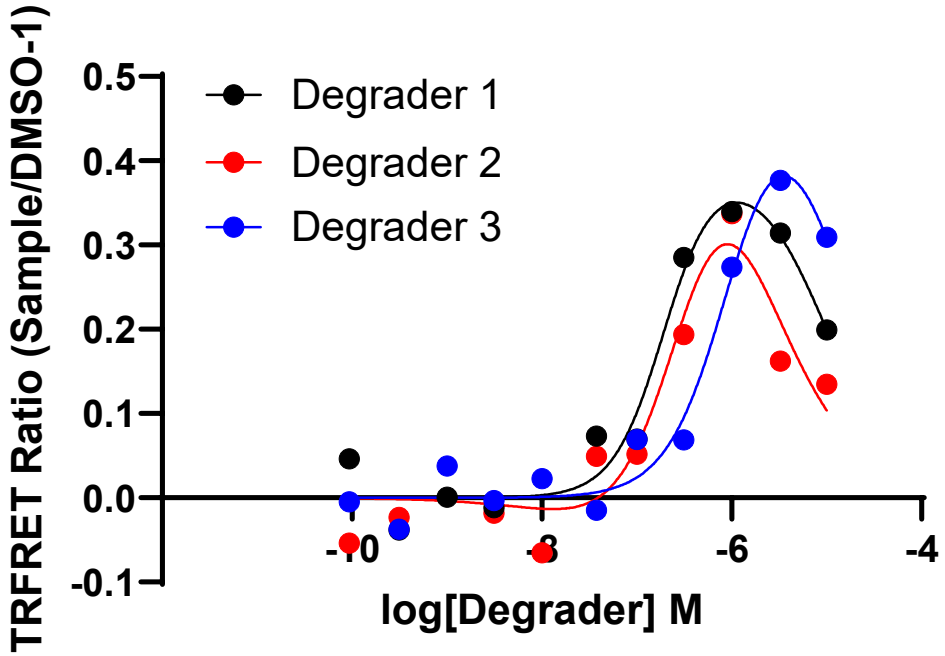
- Rapid analysis and rank order of degraders engaging both target protein and ligase
- Reduced reagent consumption, >5-fold increase in number of compounds/assay plate

High-Throughput Format for Early Degrader Discovery

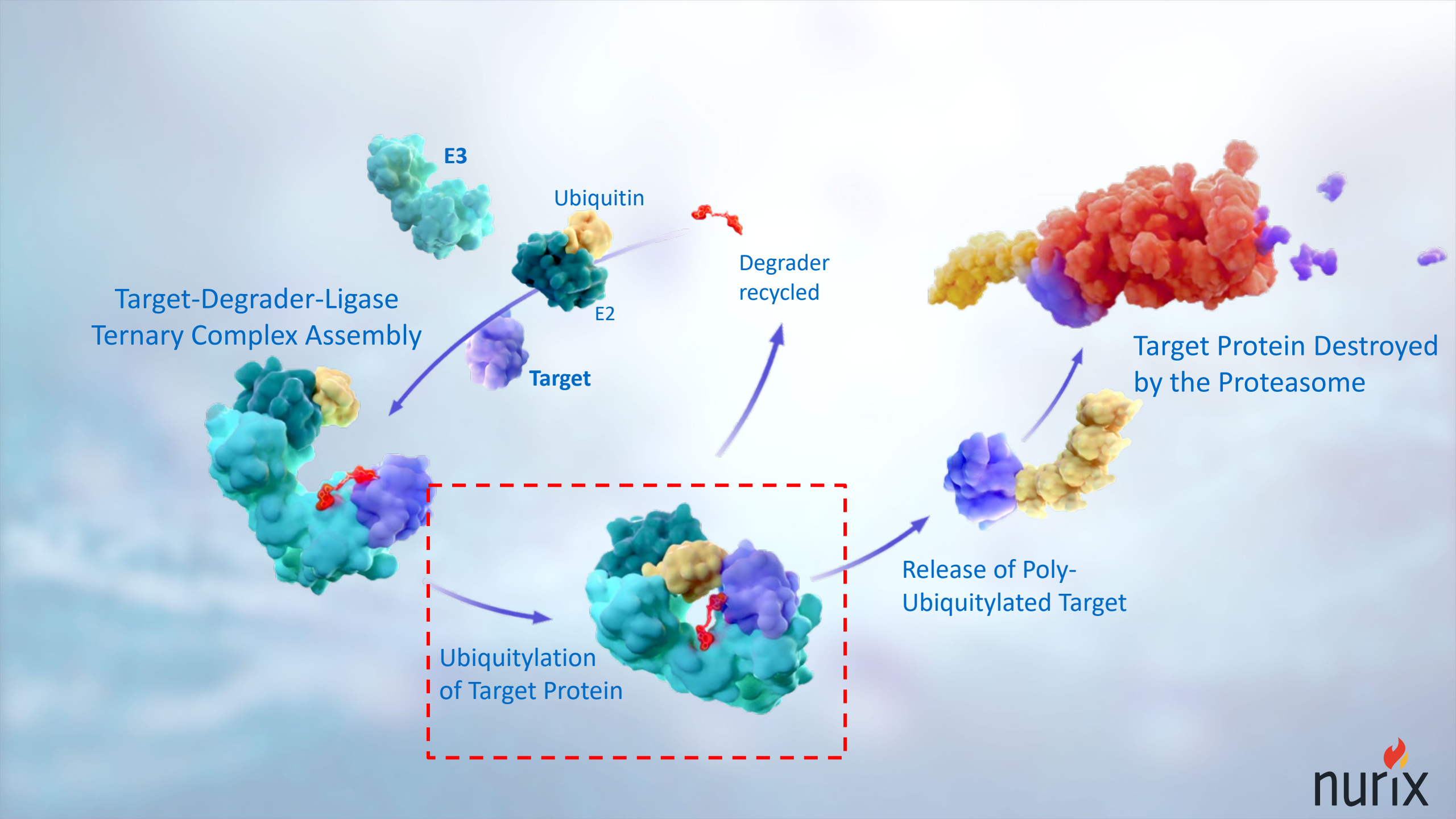
4-pt High Throughput → **11-pt Dose Response Confirmation**



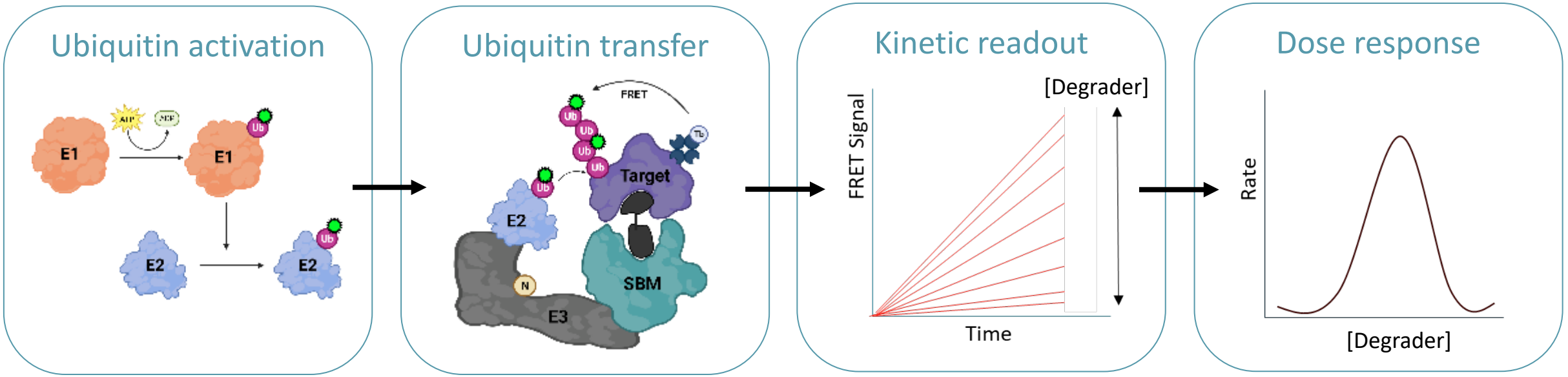




- 422 bivalent molecules profiled for ternary complex formation
- No control molecule available to optimize assay before screen
- Rapidly identify potential tool compounds for assay development and prioritize molecules for further optimization


E3
Ubiquitin
E2
Target
Degrader
recycled
Target-Degrader-Ligase
Ternary Complex Assembly
Target Protein Destroyed
by the Proteasome
Release of Poly-
Ubiquitylated Target
Ubiquitylation
of Target Protein

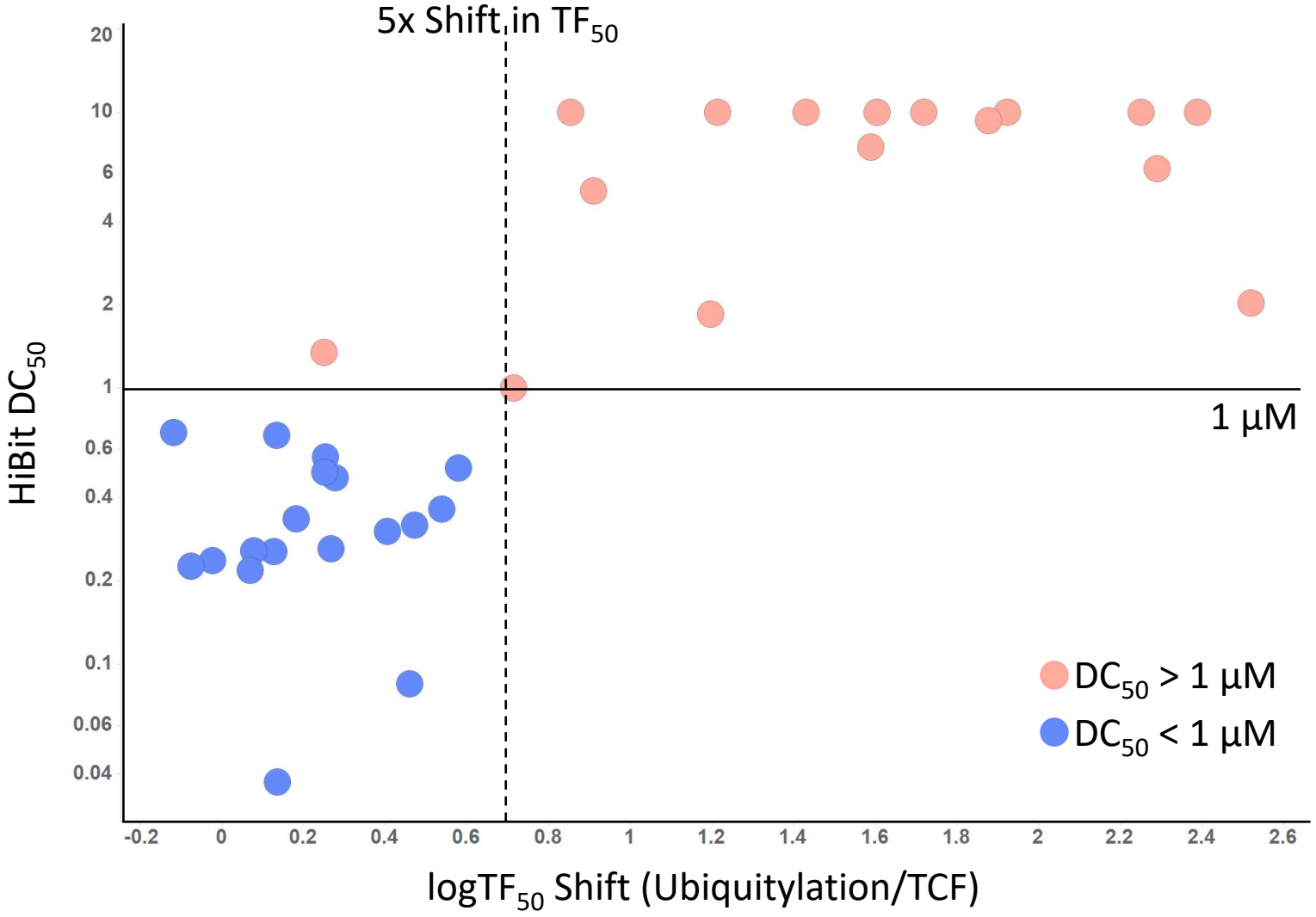
Reconstituted Ubiquitylation Activity Assay

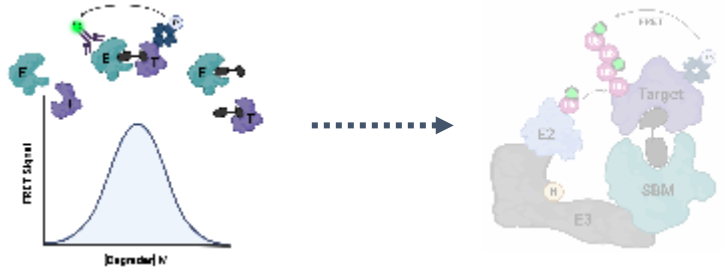


E1: Activating enzyme; E2: Conjugating enzyme; E3: Ubiquitin ligase; U: FAM-Ubiquitin;
N: Nedd8; SBM: Substrate binding module; T: Tagged substrate; Tb: Streptavidin-Terbium

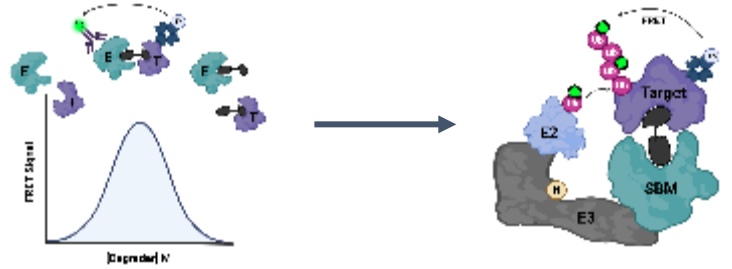
- Degrader mediated ubiquitylation of target protein
- Biotin-target/Streptavidin-terbium enables target specific TR-FRET signal
  - No contribution of auto- or off-target ubiquitylation to assay signal
  - Increase in TR-FRET is proportional to overall number of ubiquitin transferred to target

Productive Ternary Complex is Critical for Cellular Protein Degradation



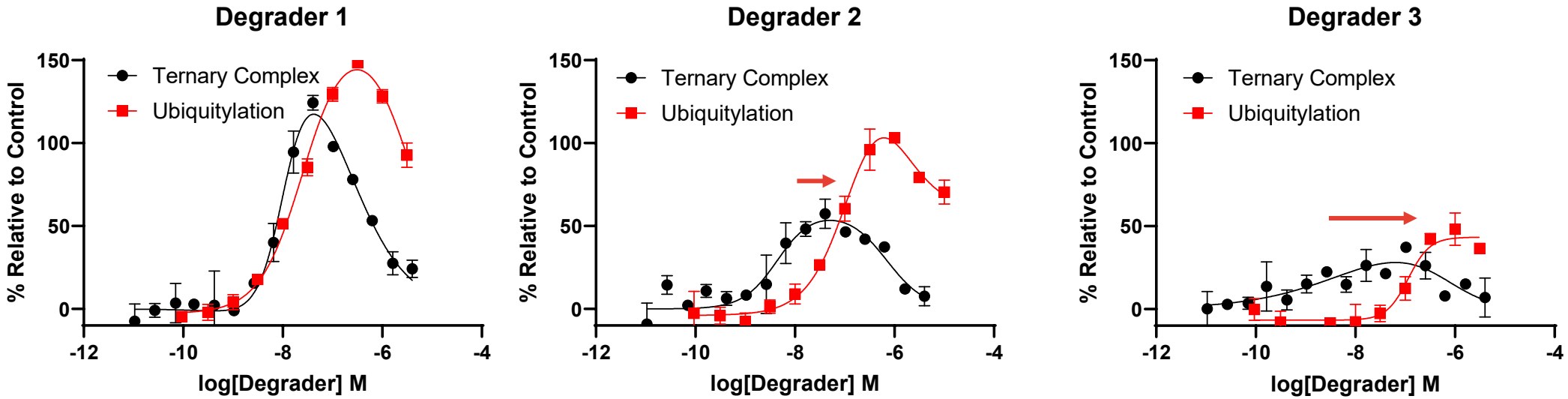


Non-Productive ternary complex- weak ubiquitylation



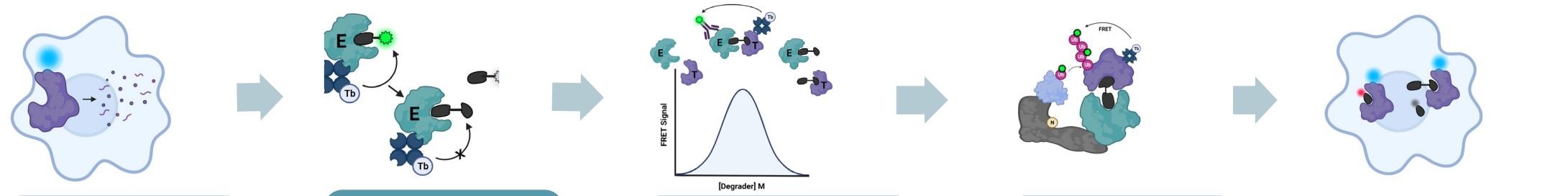
Productive ternary complex- robust ubiquitylation

Ubiquitylation Activity Assay Bridges the Gap Between TCF and Protein Degradation



Ternary Complex	TF_{50}, nM	10	≈	6	≈	2
	AUC	182		155		106
Ubiquitylation Activity	TF_{50}	30	>	80	>	117
	Vmax %	148		105		48
HiBit Degradation	DC_{50} nM	5	>>	340	>>	>10000
	Dmax %	84		73		39

Ternary Complex and Ubiquitylation Assays in the Early Discovery Pipeline



Degradation
HiBit Luminescence

Affinity Probe Displacement
TR-FRET

Ternary Complex Formation
TR-FRET

Ubiquitylation Activity
TR-FRET

Target Engagement/ Permeability
NanoBRET

If HiBit Primary Screen Shows No Protein Degradation

Series 1 → **Optimize Permeability**

Series 2 → **Optimize Vector Attachment or Linker Length**

Series 3 → **Optimize Vector Attachment or Target Binder Affinity**

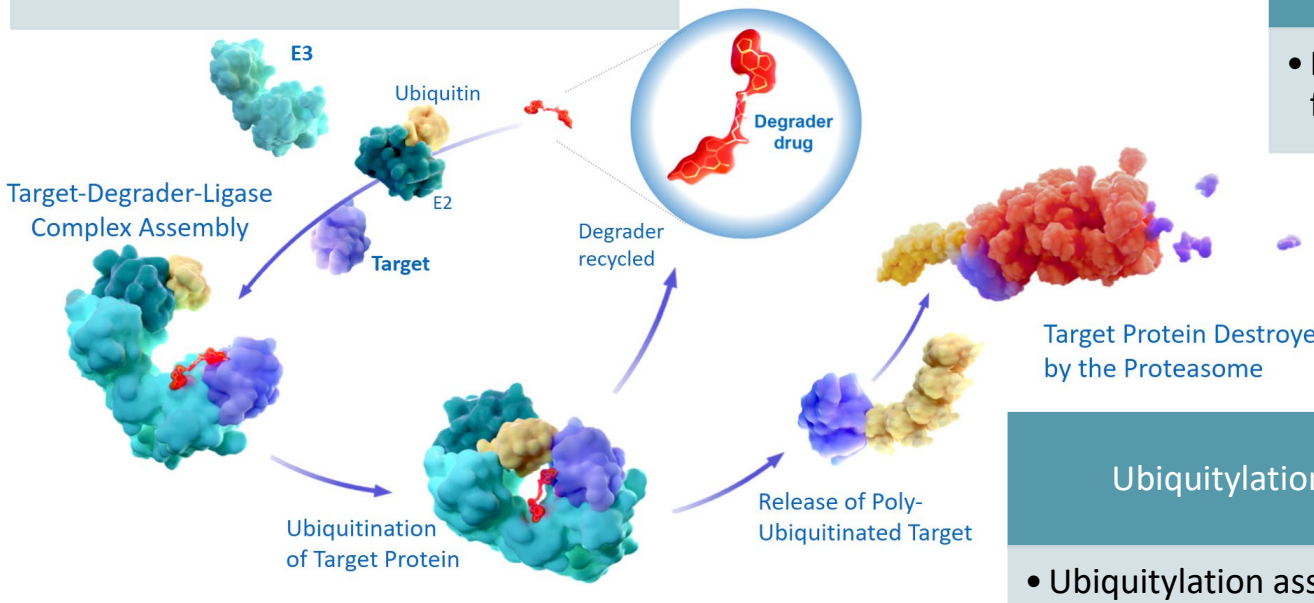
Assay Toolbox for Degrader Characterization

Probe Displacement TR-FRET

- Confirm activity of target and ligase binder motifs when connected by a linker

Ternary Complex TR-FRET

- Assay to assess simultaneous engagement with both target and ligase
- Scalable platform for degrader profiling



Protein Degradation HiBit Luminescence

- Primary screening modality for protein degradation

Ubiquitylation Activity TRFRET

- Ubiquitylation assays better predict cellular degradation in a biochemical setting
- Describe ternary complex productivity

Thank You!