Unlocking Targeted Protein Degradation

Kristin M. Riching, Lindsay Mesure, Sarah Mahan, Elizabeth A. Caine, Cesear Corona, Danette L. Daniels, and Marjeta Urh

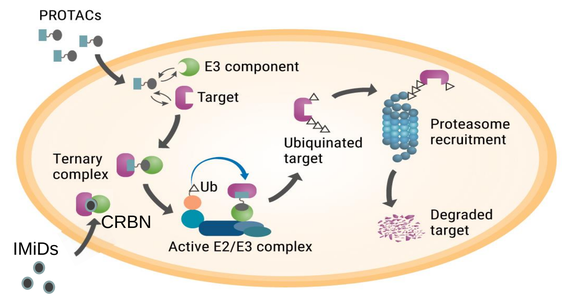
Promega Corporation, 5430 East Cheryl Parkway, Madison, WI 53711

1. Introduction

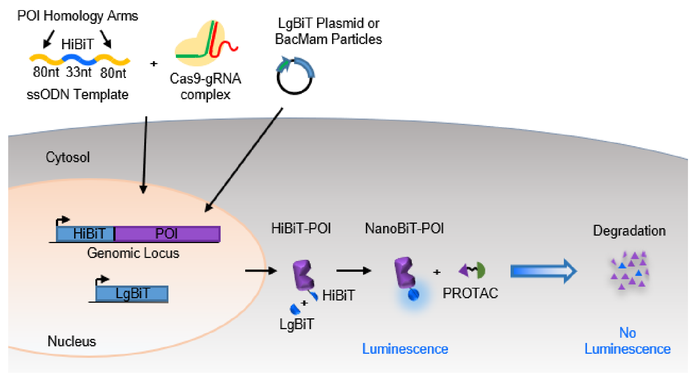
Targeted protein degradation is a promising new therapeutic strategy consisting of small molecules, most commonly molecular glues or Proteolysis Targeting Chimeras (PROTACs), which elicit degradation of a target protein. These compounds function to bring into proximity the target protein with an E3 ligase complex component. This results in formation of a ternary complex which serves to ubiquitinate and degrade the target protein via the ubiquitin proteasomal pathway. Significant challenges persist to characterize the cellular mechanism of action and the highly dynamic degradation responses induced by these compounds. Here we present a live-cell, luminescence-based technology platform, combined with CRISPR/Cas9 endogenous tagging, with these capabilities. Tagging of target proteins with the small peptide, HiBiT, which has high affinity for and can complement with the LgBiT protein to produce NanoBiT luminescence, allows for sensitive detection of endogenous protein levels in living cells and can also serve as a BRET energy donor to study protein:protein or protein:small molecule interactions required for successful degradation. We demonstrate the ability to quantitate key degradation parameters for compound triaging and ranking including rate, Dmax, and Dmax50. We further confirm mechanism by monitoring the kinetics of induced ternary complex formation and target ubiquitination using either a PROTAC or molecular glue. Finally, we highlight the ability of these tools to identify the key cellular mechanisms underpinning the dramatic improvement in degradation potency and kinetics of SIM1, a novel trivalent degrader, compared to the parent bivalent MZ1 PROTAC. Together, these approaches expand the capabilities for understanding degrader efficacy in live cells as well as identifying next generation protein degradation therapeutic targets.

2. Targeted Protein Degraders: PROTACs and Molecular Glues

Hijacking the UPS with PROTACs and Molecular Glues

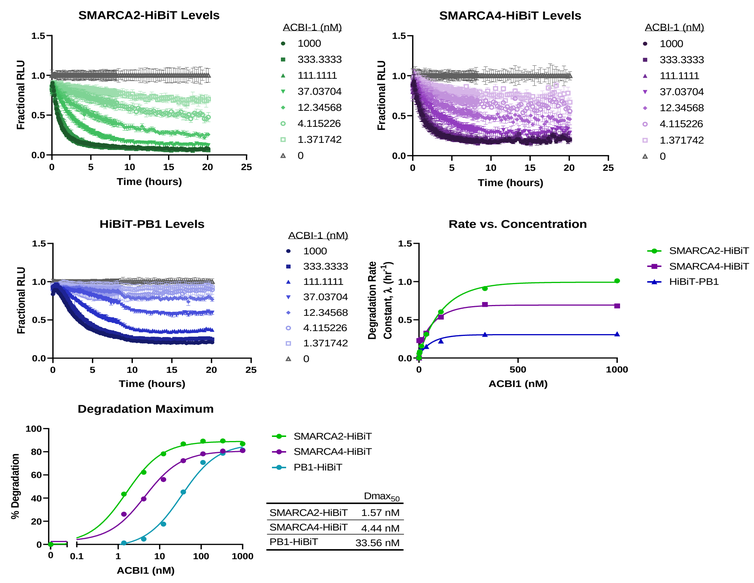


HiBiT CRISPR Tagging Strategy and Experimental Approach



3. PROTAC-Induced Live Cell Degradation Kinetics

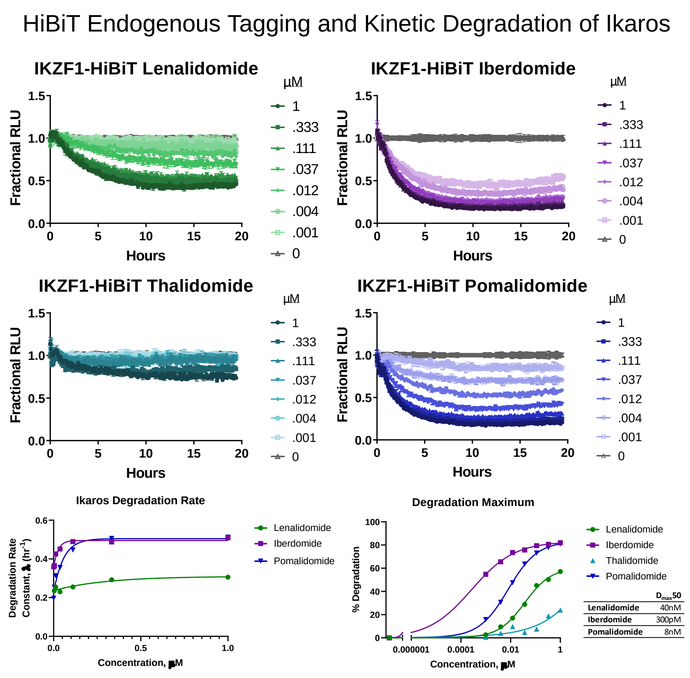
HiBiT Endogenous Tagging and Kinetic Degradation of BAF Complex Proteins: SMARCA2, SMARCA4, and PB1



- Complete cellular degradation profiles determined with continual luminescent reads on GloMax Discover
- Degradation rate, Dmax, and $Dmax_{50}$ determined to rank compound potency across the different targets

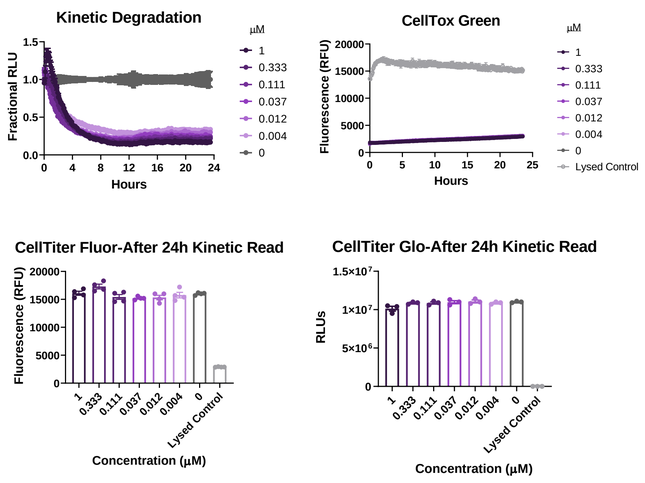
4. Molecular Glue-Induced Live Cell Degradation Kinetics

HiBiT Endogenous Tagging and Kinetic Degradation of Ikaros



- Observe degradation of molecular glue targets, and can calculate degradation rate, Dmax, and $Dmax_{50}$ to determine differences in compound potency
- Can be used for counter-screening PROTACs that have a glue handle

5. Multiplexing Ikaros degradation by Iberdomide with cell viability



- Confirm loss of signal (degradation) is not due to impacts on viability
- Kinetics can help distinguish target degradation that may later lead to toxicity
- Different viability assays to measure different stages of toxicity

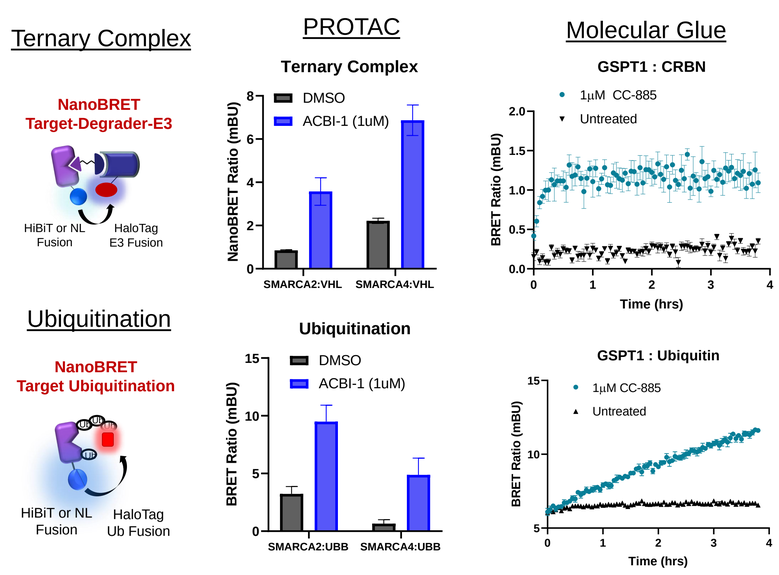
Kinetic Multiplex:

- CellTox-Green: membrane integrity

Endpoint Mulitplex:

- CellTiter-Fluor: active cellular proteases
- CellTiter-Glo: cellular ATP levels

6. Live Cell NanoBRET Ternary Complex and Ubiquitination Monitoring



- Endpoint or kinetic measurement of ternary complex formation or ubiquitination in live cells
- Compatible with PROTAC and molecular glue compounds
- Use endogenous HiBiT-fused target protein, or ectopic expression
- Kinetic analysis allows for understanding of cellular ternary complex stability

7. Trivalent BET Family PROTAC Improves Degradation Potency and Kinetics

Trivalent PROTAC, SIM1

- Trivalent SIM1 PROTAC simultaneously engages both bromodomains of BET family proteins with VHL
- More potent and faster degradation kinetics observed with trivalent SIM1 compared to bivalent MZ1 for all BET family members, as shown for BRD2.
- Imaide, S., Riching, K.M., Makukhin, N. *et al. Nat Chem Biol* **17,** (2021).

8. Trivalent SIM1 Forms Long-Lived, Stable Ternary Complex for Robust Ubiquitination

Ternary Complex

Ubiquitination

- SIM1 enhances ternary complex formation and ubiquitination kinetics.
- Most prolonged residence time with SIM1 and dependent on ability to engage VHL (cis-SIM1 does not bind VHL), indicative of cooperativity
- Bivalent MZ1 also shows longer residence time when VHL is available for binding, however, not as long as SIM1.
- SIM1 enhances degradation via avidity and cooperativity

9. Conclusions

Differentiating cellular technologies to study key processes in PROTAC and Molecular Glue-mediated degradation for rapid profiling and rank-ordering of compounds, and monitoring of phenotypic response

HiBiT and NanoLuc Technologies:

- Live cell kinetic degradation
- Amenable for use with CRISPR to study endogenous proteins
- Allows for quantitation of key degradation parameters

NanoBRET Technology:

- Monitoring dynamic pathway interactions and signaling mechanisms in live cells
- Follow induced interactions with E3 ligase components and target ubiquitination
- Residence time experiments can inform on intracellular ternary complex cooperativity
- Provides mechanistic understanding of degradation kinetics

Corresponding author: Kristin.riching@promega.com