Leveraging E2 Ligases for Induced Proximity and Modulation of Novel Cancer-Relevant Targets and Neosubstrates

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ABSTRACT

Ubiquitin-conjugating enzymes (E2s) govern ubiquitin chain topology and flux through the ubiquitin–proteasome system, thereby shaping proteostasis, DNA-damage responses, and oncogenic signaling. Although targeted protein degradation (TPD) has largely focused on E3 ligases, convergent evidence from recent chemical biology and oncology studies indicates that E2s are druggable nodes with dual therapeutic potential for reprogramming using bifunctional and molecular glue degraders. Here, we demonstrate that E2s can act as a recruitment engine for proximity-induced degradation when E3 access or cooperativity is limiting for a distinct set of targets. We introduce a first-in-class, selective, small-molecule–based bifunctional degrader platform that exploits E2 recruitment to degrade oncology-relevant targets, including nuclear receptors and kinases. In biochemical and cellular systems, the ligand engages its E2 target with high selectivity, induces proximity through ternary complex formation and degrades the protein of interest in a proteasome- and Cullin-dependent manner. We validated our approach by designing bifunctional degraders of ERα, coupling ERα and E2 ligands with short linkers, and demonstrating target degradation in MCF7, T47D, SH-SY5Y, and K562 cell lines. We demonstrate early degradation by 6 hours and maximal degradation at 24 hours with a D_{max} of 85% and DC_{50} of 83nM. Further, by conjugating 4 kinase inhibitor scaffolds with 2-6 linker designs, we screened the proteome to cover almost 500 potential kinase targets in K562 and MCF7. We demonstrated dose-dependent degradation of dozens of kinases, including kinases of potential therapeutic interest such as SYK, FYN, and MAPK2. We observed dramatically different sensitivity to degradation across the cell lines tested, and degradation activity was more robust at the 24h timepoint compared to 5h. Collectively, these findings validate E2 ligases as functional recruiters for TPD, expanding the degrader toolbox beyond canonical E3 ligases and establishing a complementary, generalizable framework for therapeutic development.

COMPONENTS OF THE UBIQUITIN PROTEASOMAL PATHWAY

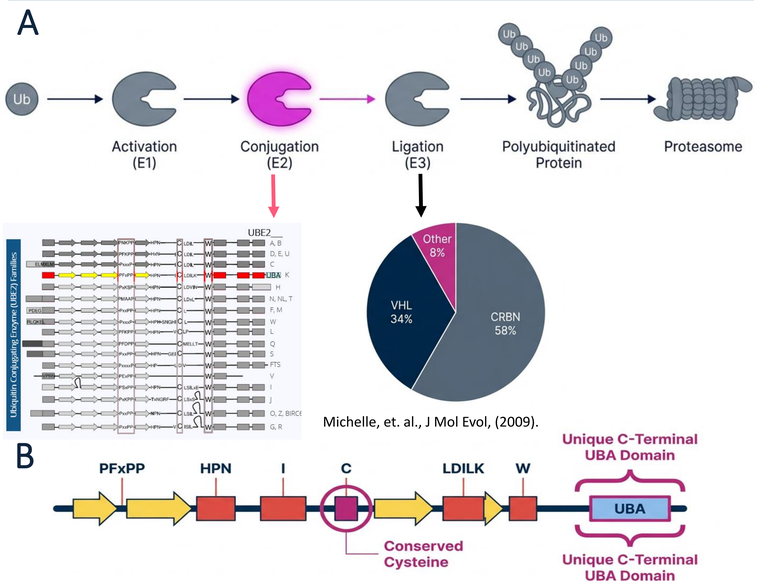


Figure 1: (A) Schematic representing the Ubiquitin Proteosomal Pathway of protein homeostasis. Conventional Bifunctional Degraders (BFDs) are designed based on targeting the E3 enzymes. The novel modality described here, leverages E2 enzymes. The human genome codes for 40 to 50 E2s. (B) We describe a unique E2 namely UBE2K, structurally distinct due to its C-terminal UBA domain.

CONFIRMATION OF UBE2K SELECTIVITY AND CELLULAR PHARMACOLOGY OF E2 LIGANDS

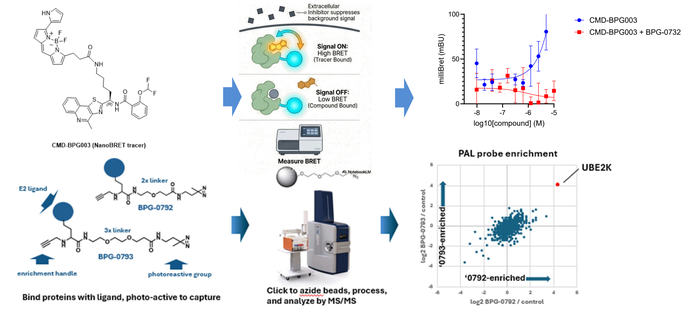


Figure 2: (A) NanoBRET Assay demonstrating competitive binding of UBE2K ligands with a tracer molecule, the data conforms cellular pharmacology and engagement of UBE2K. (B) Photoactivated affinity-based probes designed based on E2 scaffold, confirming selectivity of these scaffolds for UBE2K by mass spectrometry.

DEMONSTRATION OF ERα DEGRADATION USING 2 DISTINCT ERα LIGANDS, LEVERAGING E2 LIGASE

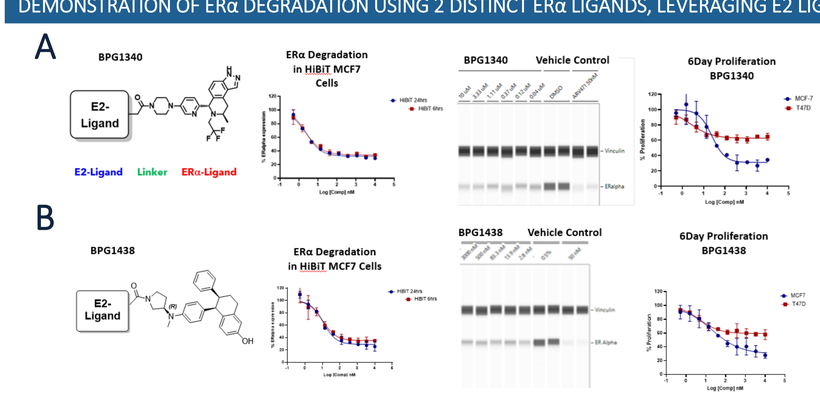


Figure 3: Plug and Play approach: Demonstrating ERα degradation using 2 different Protein of Interest Ligand. Both ligands demonstrate early and potent degradation of target (ERα) at 6 hrs, confirmed both by HiBiT assay and Protein Simple. Both ligands also demonstrate potent antiproliferative effects in MCF7 and T47D breast cancer cell lines.

LEAD OPTIMIZED BFDS OF ERα DEMONSTRATE DEGRADATION & CELL PROLIFERATION EFFECTS

A

Compound ID	ERα Degradation Parental MCF7 Cells (24 h) Degradation (% @ Concentration)	6d Proliferation (EC50, Emax) MCF7	 T47D
BPG-1377	68% @ 3 µM, 78% @ 500 nM, 81% @ 83.3 nM, 77%@13.89 nM, 74% @ 2.31 nM	2.6 (71%)	0.35 (39%)
BPG-1386	68% @ 3 µM, 72% @ 500 nM, 76% @ 83.3 nM, 73%@13.89 nM, 72% @ 2.31 nM	8.5 (62%)	3.8 (38%)
ARV471	78% @50nM	7.8 (78%)	0.82 (57%)

B

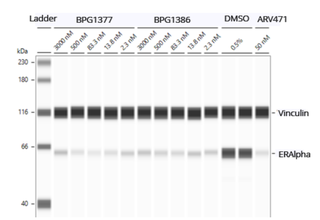
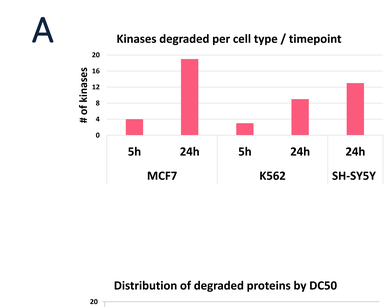
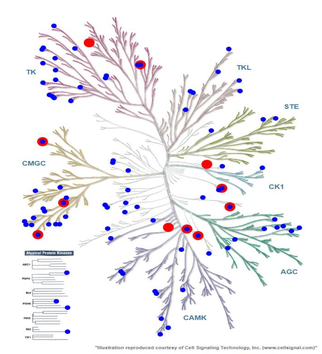


Figure 4: (A & B) Ligands optimized for DMPK and ADME parameters demonstrate potent degradation and antiproliferative effects in breast cancer cell lines.

DISCOVERY OF NOVEL KINASE TARGETS FOR DEGRADATION LEVERAGING E2 LIGASE BFDS



DC50 for degraded kinases are most commonly in the high nM/ low µM space with these non-optimized scaffolds.



Kinome tree coverage[5] of selected TAE684-based degraders: SK-3-91, a CRBN-based PROTAC[1] and BPG-1226 E2-based degrader demonstrate differing kinase family activity.

[5]Eid et al. BMC Bioinformatics, 2017. KinMap: a web-based tool for interactive navigation through human kinome data

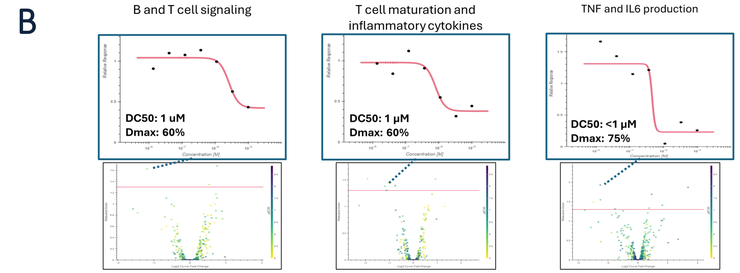


Figure 5: (A & B) Examples of oncology relevant kinases degraded by Dabrafenib-based E2 designs. Volcano plots of fold change and relevance score are provided for context of the global data.

CONCLUSIONS

- E2 Scaffolds and proximity induction enables an orthogonal means of modulating protein homeostasis and function, thereby potentially opening up a novel repertoire of druggable protein targets.
- E2 scaffolds provide a modular platform for establishing “Targeted Protein Degradation” for novel targets.
- E2 scaffold based Bifunctional Degraders are drug-like and exhibit favorable DMPK and ADME properties.
- BPG-1377 and BPG-1386 will be tested in *in vivo* proof of concept studies in WT and CRBN KD cells to confirm CRBN independent mechanism of degradation of ERα.
- Novel kinase targets will be validated *in vitro* and optimized for *in vivo* PK and PoC studies.