MOPED™ Emerald : A Novel Platform for Molecular Glue Discovery used to Identify CRBN-based Degraders for IKZF3

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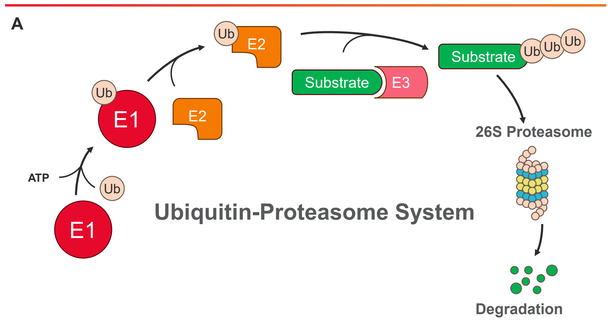
Background

- Molecular glues are small molecules that can stabilize or strengthen protein-protein interactions (PPI).
- Molecular glues have properties to be safe, oral drugs (i.e. low molecular weight, selective through PPI dependence).
- Discovery of novel molecular glues is often difficult, with most examples to-date being serendipitous.
- Current strategies for novel molecular glue discovery have limitations (e.g. resource intensive, lack ability for high-throughput screening).

Key Findings

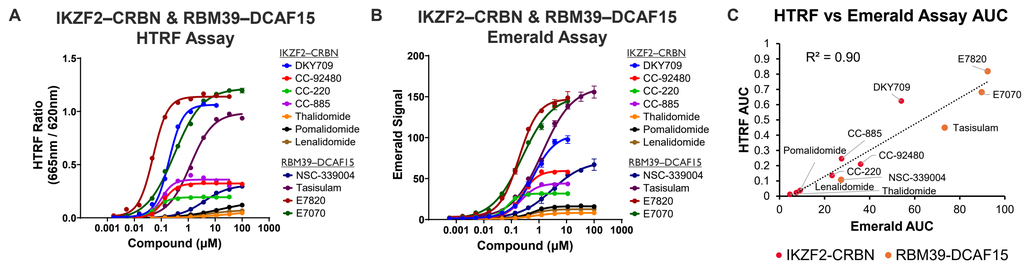
- Developed MOPED™ Emerald – a novel MOlecular Proximity Enabled Detection assay and platform for high-throughput molecular glue discovery.
- Emerald assay validated and correlates with HTRF assay results for known molecular glue systems.
- Emerald assay shown to have greater sensitivity for detecting ternary complex formation compared to traditional HTRF-based assays.

Introduction



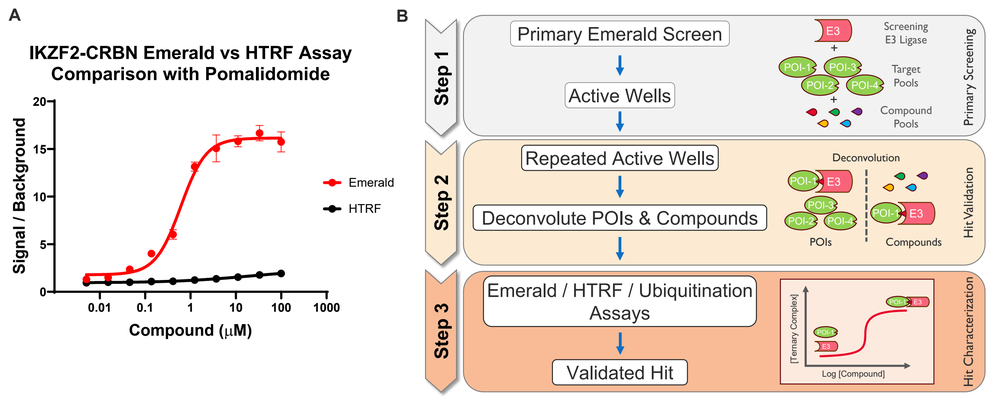
(A) The ubiquitin–proteasome system (UPS) is an ATP-dependent protein degradation system in cells. In general, there are two types of small-molecules that can use the UPS to selectively degrade proteins. **(B)** Heterobifunctional compounds (> 500 Da MW) contain two active warheads, binding E3 and Target Protein, tethered by a linker and are prone to "Hook" effects due to binary warhead affinities. **(C)** Molecular glues are smaller molecules (< 500 Da MW) and do not have "Hook" effects due to weak binary affinities for one or both binding partners.

Figure 1. Emerald Assay correlates with HTRF ternary complex assay



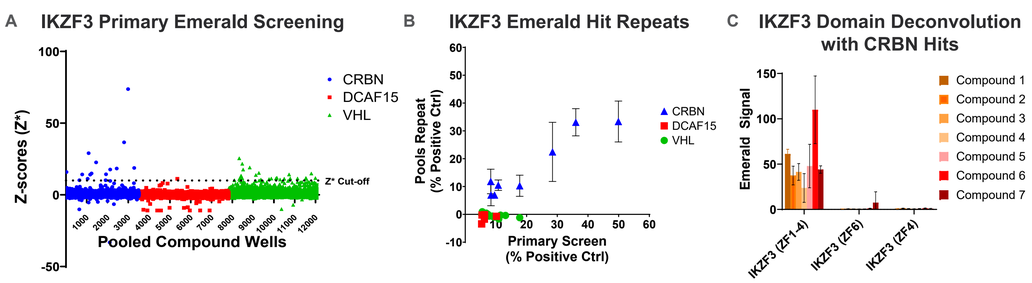
(A & B) HTRF and Emerald ternary complex binding assays with known molecular glue degraders for IKZF2–CRBN and RBM39–DCAF15. **(C)** Correlation plot for area under the curve (AUC) analysis from Emerald and HTRF assays with IKZF2–CRBN and RBM39–DCAF15. A strong correlation (R^2=0.9) is observed between the two assay formats.

Figure 2. Emerald Assay has greater sensitivity for detecting ternary complex formation and is amenable for high-throughput screening



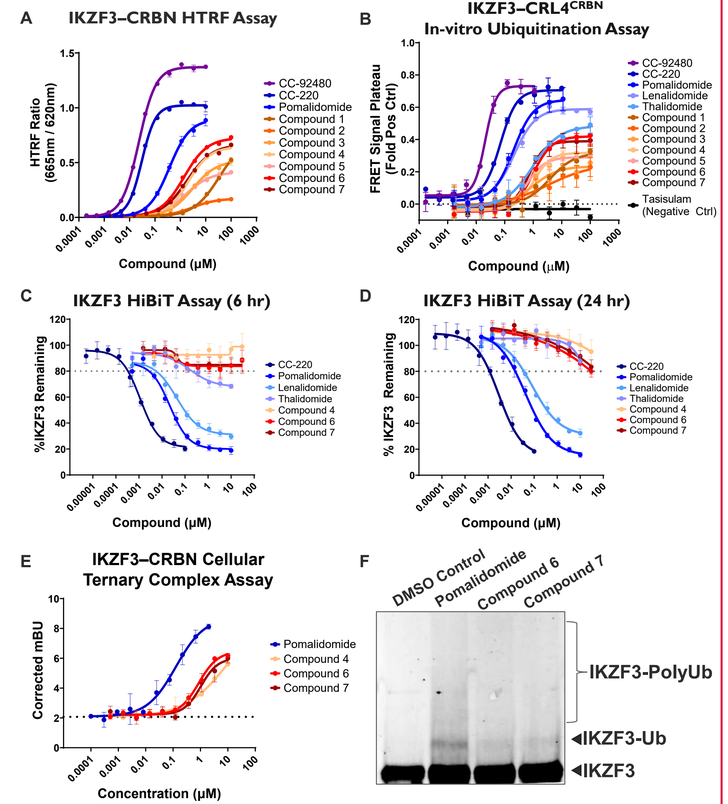
(A) Signal to Background (DMSO) comparison of Emerald and HTRF assays for IKZF2–CRBN with pomalidomide shows higher signal and sensitivity in the Emerald binding assay compared to HTRF. **(B)** Hit progression model and workflow for primary Emerald screening supports an efficient method for high-throughput molecular glue screening.

Figure 3. IKZF3 Primary Emerald screening identifies 7 hits with CRBN



(A) Robust Z-scores (Z*) from IKZF3 Primary Emerald Screening with CRBN, DCAF15, and VHL. **(B)** Repeat IKZF3 Emerald active wells with compound pools plotted as percent positive control for IKZF2–CRBN. Only the CRBN active wells showed repeatable activity. These compound pools were deconvoluted to seven individual compounds. **(C)** IKZF3 domain deconvolution with CRBN hits shows that Emerald activity corresponds to ZF1-4 region for IKZF3.

Figure 4. IKZF3–CRBN hits have micromolar potencies and lead to degradation in cells



(A) IKZF3 (ZF1–4) and CRBN-DDB1 HTRF ternary complex binding assays with control compounds and Emerald primary hits. **(B)** In-vitro E2-Ubiquitin discharge assay for IKZF3 and CRL4-CRBN. **(C & D)** IKZF3 HiBiT assay at 6 hr and 24 hr timepoints. Dotted lines represent 80% level of IKZF3 protein remaining. **(E)** IKZF3–CRBN NanoBRET cellular ternary complex. Dotted line represents base line background signal for assay. **(F)** IKZF3 Western blot for Anti-FLAG IP samples from cells treated with DMSO, pomalidomide, and compounds 6 and 7.

Conclusions

- Identified 7 primary hits for IKZF3 with CRBN from a library of 460,000 compounds using our MOPED Emerald platform.
- IKZF3 Emerald hits validated to form ternary complexes with CRBN and shown to have ubiquitination activity in-vitro.
- Several hits shown to form ternary complexes with CRBN in cells, resulting in IKZF3 ubiquitination and degradation.

References

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