HIGH-THROUGHPUT SCREENING OF MOLECULAR GLUES USING SPECTRAL SHIFT TECHNOLOGY: A COMPREHENSIVE APPROACH FOR EARLY-STAGE DRUG DISCOVERY

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Abstract

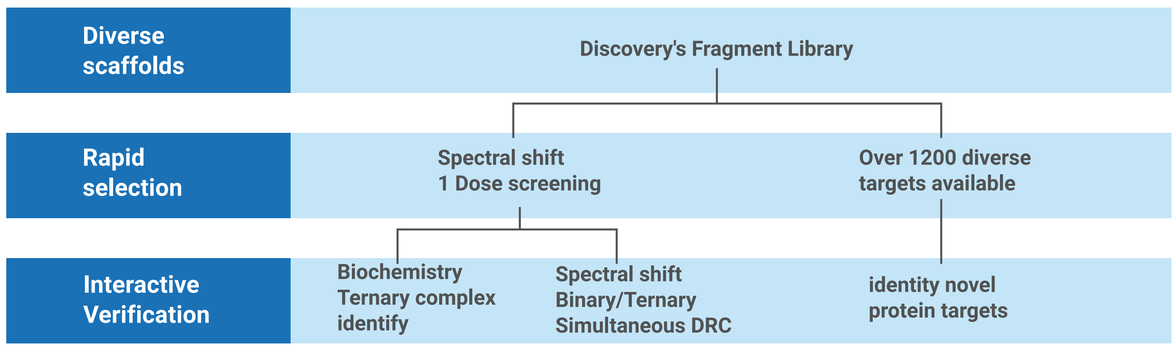
Fragment-based drug design (FBDD) has emerged as a powerful strategy in drug discovery, particularly for identifying novel scaffolds and binding sites for challenging targets. We have employed a combination of Spectral Shift and TR-FRET technologies to discover new molecular glue scaffolds. Our fragment library is diverse and includes a rich variety of E3 ligases from different species, providing a sustainable and versatile screening platform.

Our key strengths lIe in the high-throughput screening of affinity fragments using Spectral shift technology. We further validate the formation of ternary complexes through TR-FRET.

Subsequently, we refine our selection by screening for molecules with high a-values using Spectral Shift technology again. Ul timately, this integrated approach has led to the discovery of novel molecular glue scaffolds, showcasing our innovative and effective strategy in fragment-based drug design.

Fragment-Based Screening (FBS) Strategy

A



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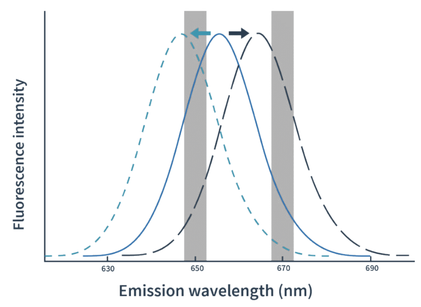
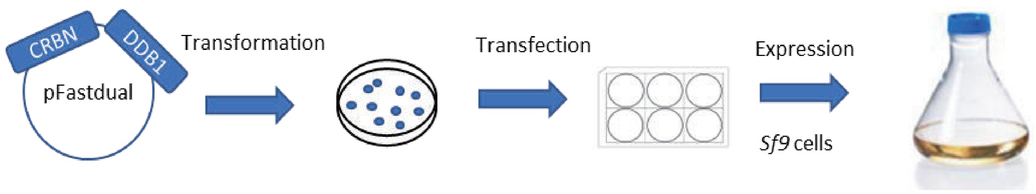


Figure 1. Fragment-based Drug Discovery (FBDD) cascade for Molecular glue. A.Molecular glue screening cascade from a 1308-fragment llbrary using Spectral Shift. induced Proximity fragment binding was confirmed by DRC confirmation and TR-FRET assays.
B. Spectral Shift technology used for the primary binder sereen and confirmation. Dianthus uses a dual-emission spectral shift measurements, where two separate detectors collect the light at two distinctive wavelengths: 650 and 670 nm in parallel, shown here by the grey bars. The ratio 670nm/650nm is then plotted against the ligand concentration used in the assay and use a Kd fit model to derive a Kd.

Production of Active CRBN-DDB1

- **CRBN/DDB1**, A binary complex, were co-expressed in *sf9* cells and purified by Ni-NTA column and followed by size exclusion chromatography.
- **Cloning and Expression**



- **Purification**

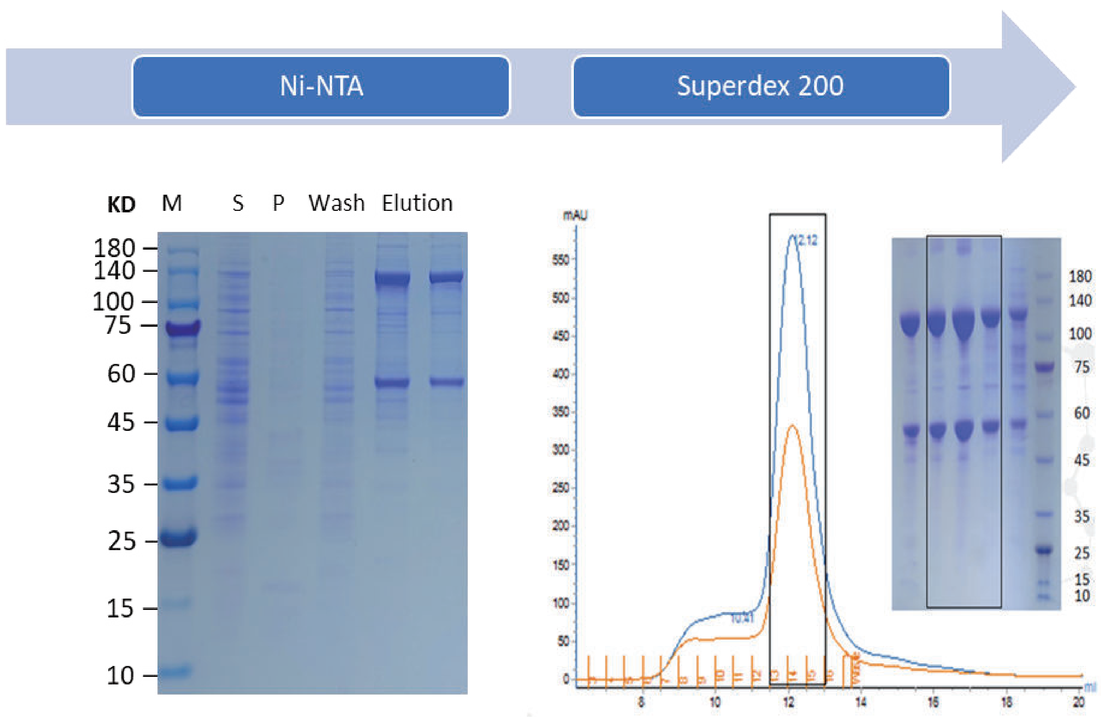


Figure 2. Purification of stable and active CRBN/DDB1 complex.

High-Throughput Automation Screening Process

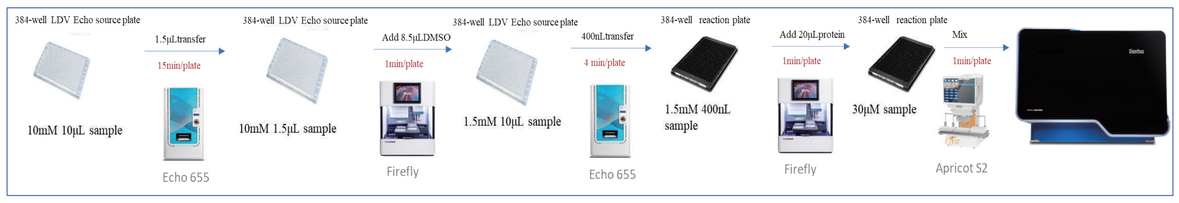
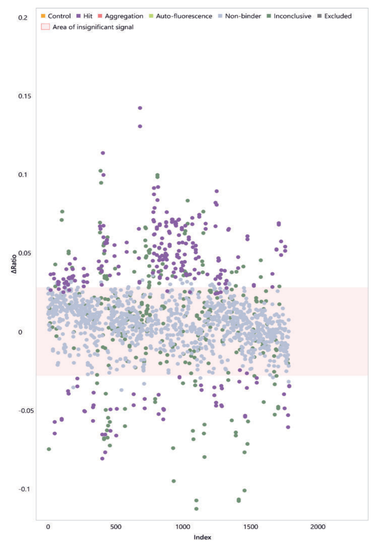


Figure 3: High-Throughput Automation Screening Process.From sample prep to lead validation in 2h with a fully automated workstation

Single-Dose Experiment for CRBN Primary Screen

A



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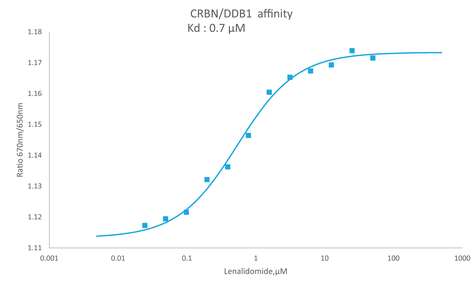

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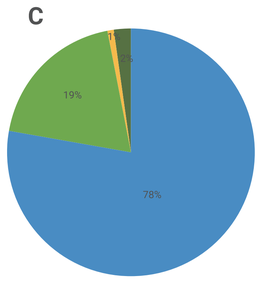
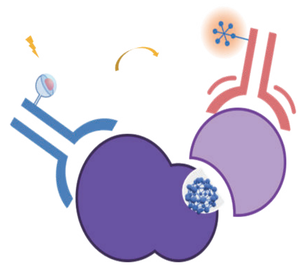


Figure 4: A)Hit screen by Spectral Shift Technology ;B) Spectral shift dose–response curves for Lenalidomide. C) Summarizing the proportion of binders after primary screen (19%).

CRBN&NEK7 Ternary complex

A



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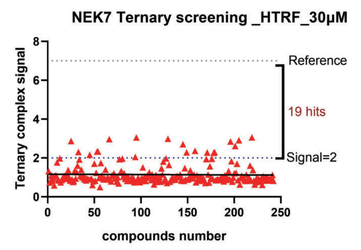

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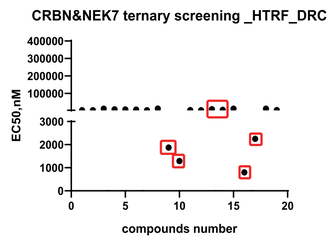

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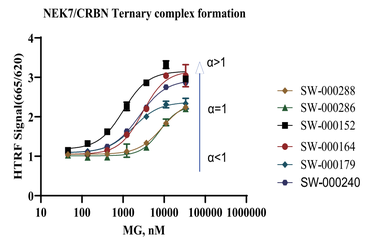
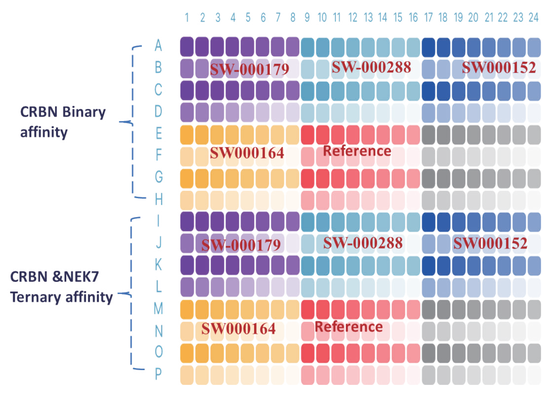


Figure 5: A) Principle of Ternary Complex Formation Experiment. B) Single-Concentration Screening for CRBN Binder Ternary Complex. C) Summary of CRBN & NEK7 DRC Screening Results. D)DRC Results of CRBN & NEK7 on Six Molecules

MG fragment Hit Confirmation

A



Compounds ID,μM	NEK7 Ternary HTRF		NEK7 SPS-TRIC		
	EC50	Top	Binary_Kd	Ternary_Kd	α
SW000179	1.783	2.375	0.732	0.489	1.50
SW000288	9.017	2.41	1.02	0.846	1.21
SW000152	1.025	3.157	0.845	0.17	4.97
SW000164	2.969	3.163	0.0556	0.383	0.15
Reference	0.168	4.7	1.0	0.096	10.42

B

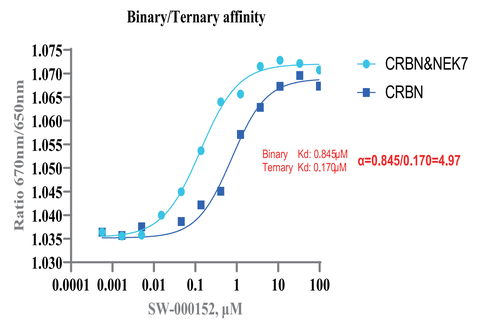


Figure 6: A) Summary table of fragment hits detected as binders (KD values) and Ternary (KD values). B) The hit demonstrates positive cooperativity in affinity for both binary and ternary complexes..

Docking Studies CRBN&NEK7 with Fragment Hit

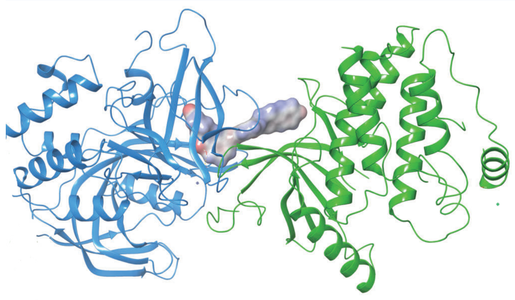


Figure 7. Docking studies of fragment hit SW-000152. In the schematic diagram, the binding interaction between CRBN, Hit, and NEK7 is depicted. The green portion represents the NEK7 protein, while the blue portion represents the CRBN protein. The fragment hit SW-000152 is positioned in the center, bridging the interaction between the two proteins.

Summary

- ICE's hit-finding team's successful MG Fragment-based Screening approach.
- ICE has produced 30 high-activity, high-purity E3 proteins, supporting the discovery of new molecules.
- The Spectral Shift technology enables rapid fragment-based single-concentration screening and subsequent single-curve affinity determination.
- Over 1,200 targets and E3 ligands have been rapidly combined and validated for activity, accelerating the discovery of new target molecules.
- A high-quality compound library with over 50,000 diverse compounds, featuring non-purine-based scaffolds, allows us to identify promising starting points for new molecular discoveries.