Building a TPD Discovery Platform for Autoimmune Therapeutics: From Biophysical Screening to *In Vivo* Validation

Qing Xue, Qian Yang, Mingying Li, Qian Wang, Lili Chai, Tiejun Bing.
ICE Bioscience, INC. Building 14, Yard 18, Kechuang 13th Street, Beijing, 100176
Email: bingtj@ice-biosci.com

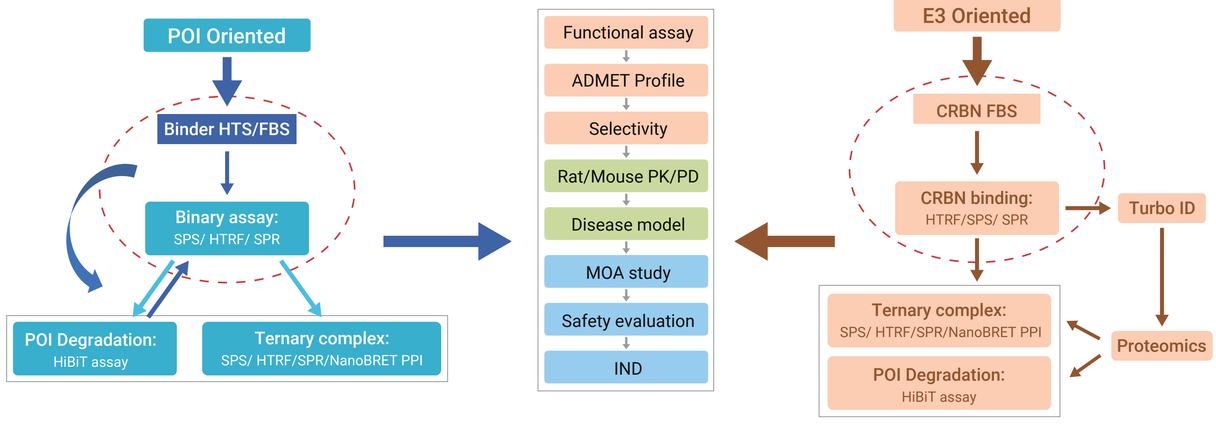
ICE Bioscience — Innovative CRO+Explorer

Abstract

Targeted protein degradation (TPD) harnesses the ubiquitin–proteasome system to eliminate disease drivers once deemed "undruggable." PROTACs recruit E3 ligases to targets via two linked warheads, while monovalent molecular glue degraders (MGDs) induce novel ligase–substrate interactions and ternary complex formation. Preclinical success in targeting VAV1, STAT6, NEK7, IRAK4 *etc*. has demonstrated remarkable efficacy in rheumatoid arthritis, atopic dermatitis and other autoimmune models, positioning TPD as a transformative therapeutic modality.

ICE's integrated platform accelerates TPD discovery through SpS-based high-throughput screening, targeted libraries and an end-to-end workflow from hit identification to *in vivo* validation. Our experience includes support for discovery of multiple MGDs and PROTACs—showcased here by discovery of a potential NEK7 MGD, and our integrated TPD platform including orthogonal biochemical and biophysics binding assays, comprehensive *in vitro* functional characterization, in depth MOA studies, *in vivo* model validation and safety evaluation. By delivering robust, actionable data at every stage, ICE empowers rapid advancement of next-generation autoimmune interventions.

Integrated Solution for TPD Drug Discovery



Spectrum Shift and Focused Library Accelerate Binder & TPD Screening

a) SpS enables binder HTS, identified potential MGDs against autoimmune targets

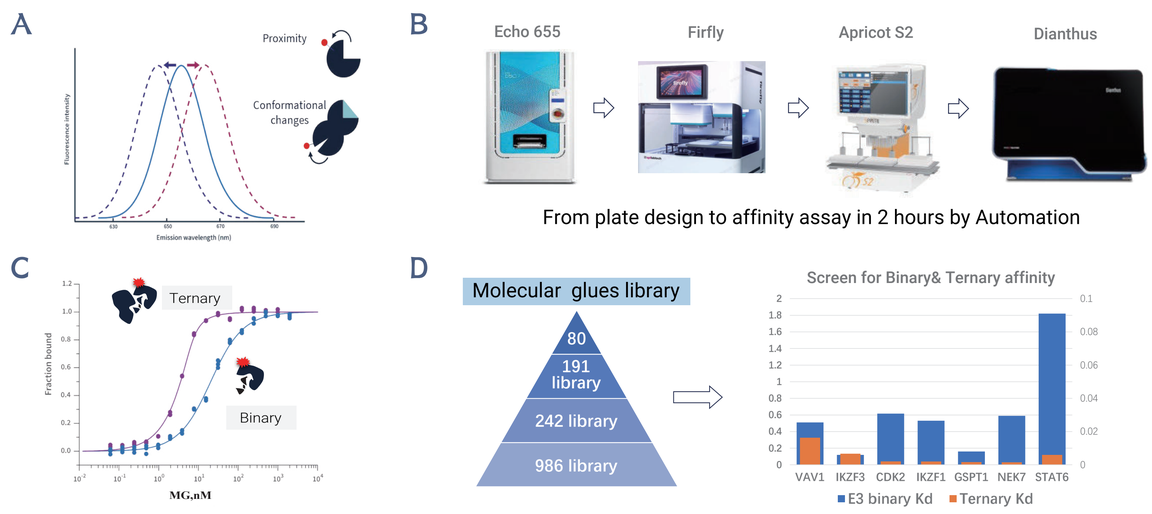
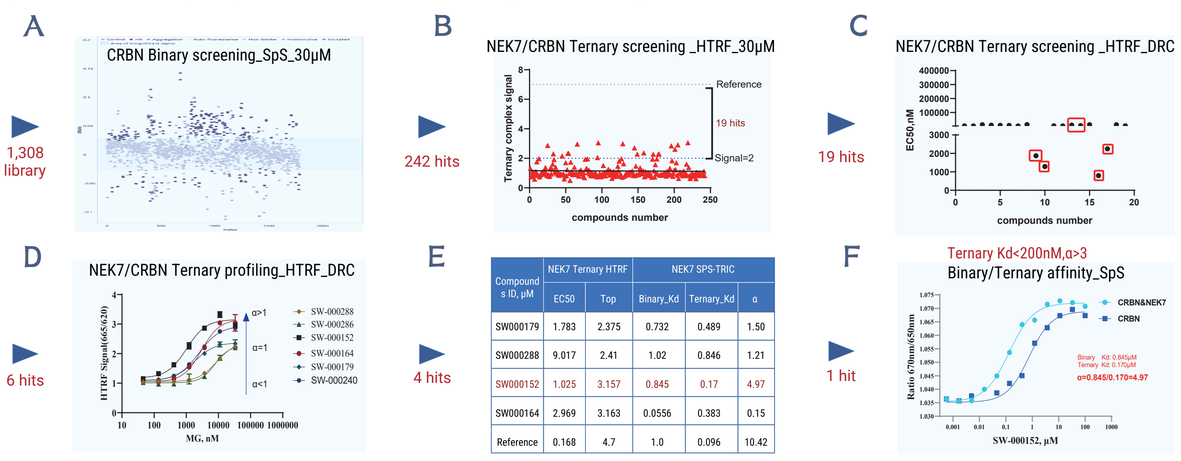


Figure 1. Spectral Shift (SpS) platform and CRBN fragment library: (A) Ligand binding induces a shift in fluorescent emission. (B) Fully automated SpS platform enables library HTS. (C) SpS profiling captures both binary and ternary binding affinities. (D) In-house CRBN fragment library accelerates MGD discovery.

b) Proof of concept SpS-HTRF MGDs screening and orthogonal validation

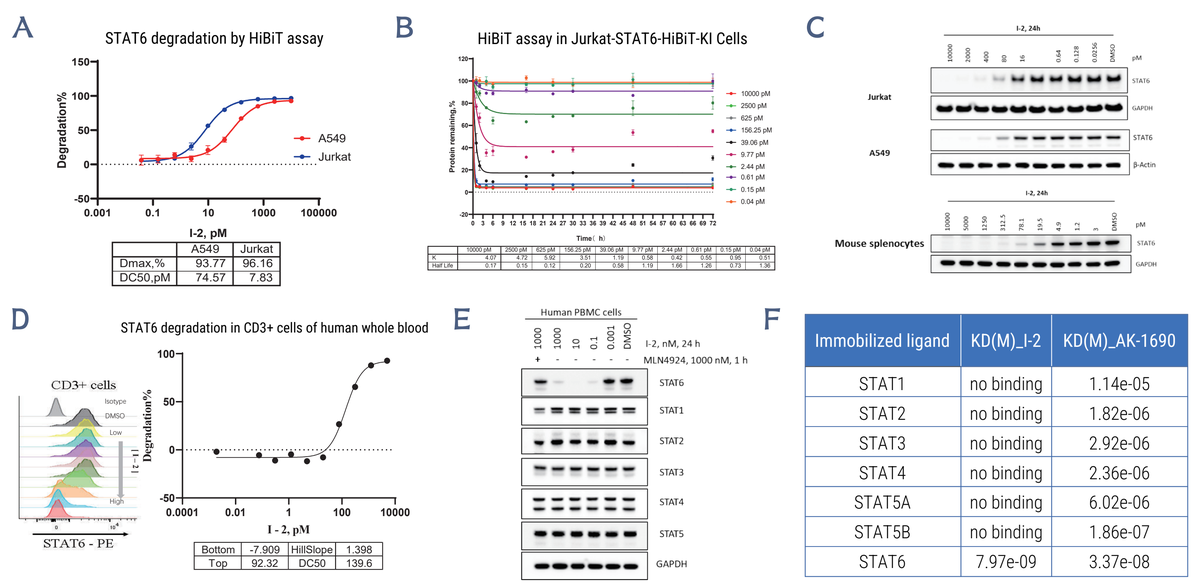


Compound s ID, μM	NEK7 Ternary HTRF		NEK7 SPS-TRIC		
	EC50	Top	Binary_Kd	Ternary_Kd	α
SW000179	1.783	2.375	0.732	0.489	1.50
SW000288	9.017	2.41	1.02	0.846	1.21
SW000152	1.025	3.157	0.845	0.17	4.97
SW000164	2.969	3.163	0.0556	0.383	0.15
Reference	0.168	4.7	1.0	0.096	10.42

Figure 2. SpS-HTRF NEK7 MGD Screening: (A). SpS screening binary binder at 30 μM (B). Single dose HTRF ternary screening. (C).(D). HTRF DRCs establish Ternary EC_{50}. (E).(F). SPS-TRIC profiling of Binary/Ternary DR.Cs. One hit validated (α > 3).

In-Depth Functional and MOA Profiling with *In Vitro* Assays

a) STAT6 Degradation Analysis by HiBiT/WB/FACS and Selectivity by WB/SPR



	A549	Jurkat
Dmax,%	93.77	96.16
DC50,pM	74.57	7.83

Bottom	-7.909	HillSlope	1.398
Top	92.32	DC50	139.6

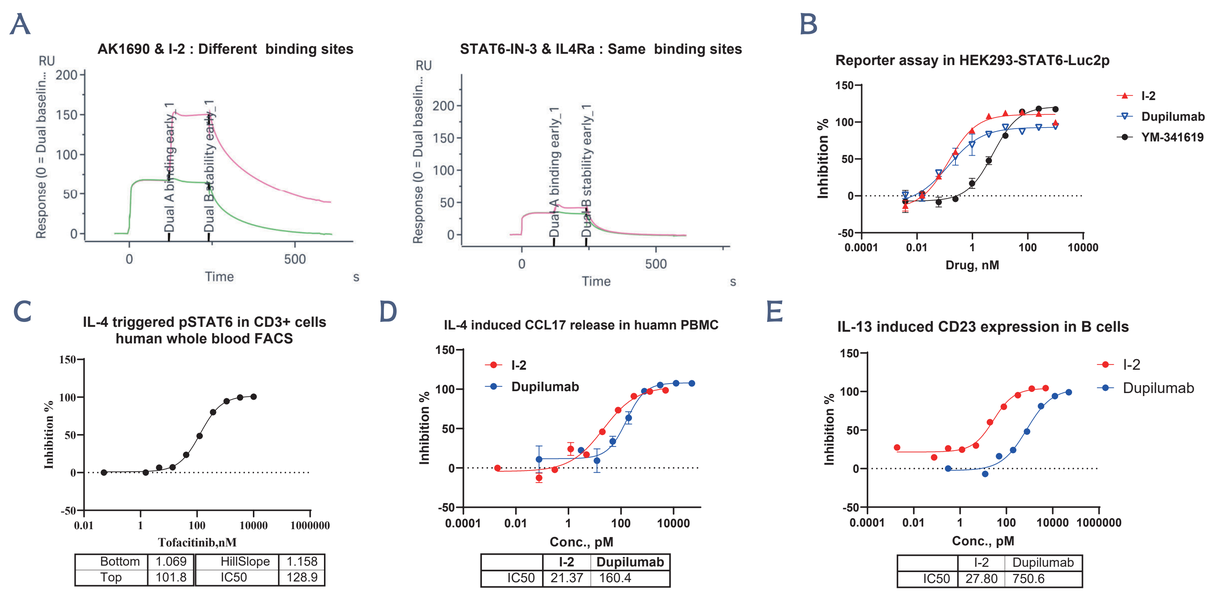
	10000 pM	2500 pM	625 pM	156.25 pM	39.06 pM	9.77 pM	2.44 pM	0.61 pM	0.15 pM	0.04 pM
K	4.07	4.72	5.92	3.51	1.19	0.58	0.42	0.55	0.95	0.51
Half Life	0.17	0.15	0.12	0.20	0.58	1.19	1.66	1.26	0.73	1.36

F

Immobilized ligand	KD(M)_I-2	KD(M)_AK-1690
STAT1	no binding	1.14e-05
STAT2	no binding	1.82e-06
STAT3	no binding	2.92e-06
STAT4	no binding	2.36e-06
STAT5A	no binding	6.02e-06
STAT5B	no binding	1.86e-07
STAT6	7.97e-09	3.37e-08

Figure 3. PROTAC-mediated STAT6 degradation and selectivity (A) HiBiT-based STAT6 degradation (B) HiBiT degradation kinetics (C) Western blot in cell lines and primary cells (D) FACS-based degradation in human T cells (E) WB selectivity profile (F) SPR selectivity profile.

b) Binding site of STAT6 PROTAC Assessed via SPR, *in vitro* functional assay



Bottom	1.069	HillSlope	1.158
Top	101.8	IC50	128.9

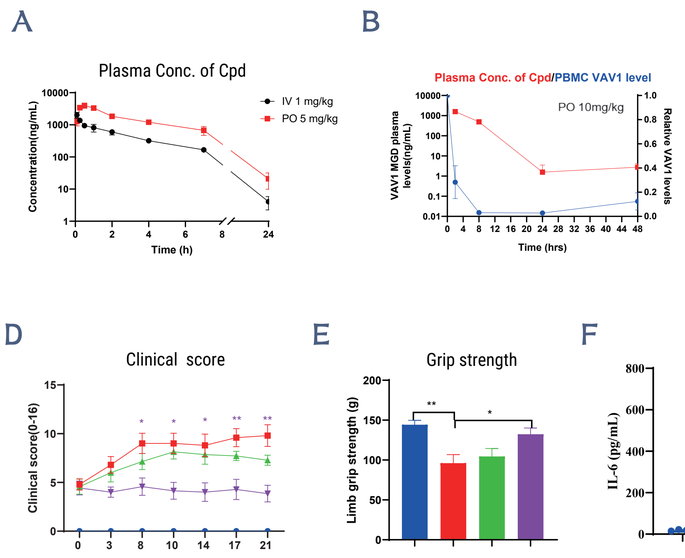
	I-2	Dupilumab
IC50	21.37	160.4

	I-2	Dupilumab
IC50	27.80	750.6

Figure 4. (A) SPR distinguishes binding sites: different-site binders produce additive signals, whereas same-site binders do not. (B) Luciferase reporter assay of STAT6 transcriptional activity. (C) FACS-based analysis of STAT6 phosphorylation following stimulation. (D) PBMC TARC secretion upon stimulation. (E) B-cell CD23 expression upon stimulation.

In Vivo disease model validation and Safety evaluation

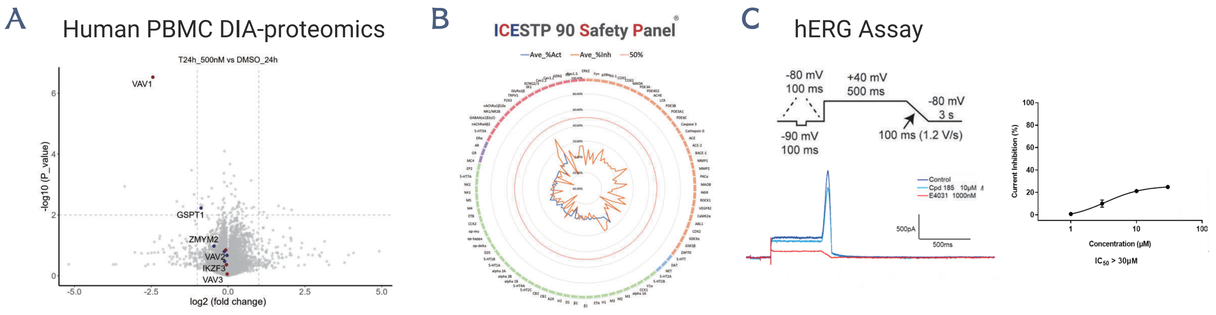
a) VAV1 MGD *in vivo* PK/PD and efficacy evaluation on Collagen-induced arthritis (CIA) mice



Parameters		ICE data	Ref
LM Stability (Human/Mouse)	t1/2 (min)	>184.79	-
	CLint (μL/min/mg protein)	<7.50	-
Plasma Protein Binding (human/mouse)	fu%	Fu%：11.82% (Human)	-
Plasma Stability (Human)	t1/2 (min)	>372.68	-
CYP Inhibition (1A2/2C9/2C19/2D6/ 3A4M/3A4T)	@10uM Inhibition%	-1.72%/10.57% / 5.62%/9.09%/ 4.98%/-2.11%	IC50>30uM
Caco-2 permeability	Papp/ER (1E-6 cm/s)	Papp A-B:19.20 Papp B-A:22.72 1.18	F%>50%

Figure 5. *In vivo* efficacy of VAV1 MGD (A)(B)PK/PD data of VAV1 MGD. (C) *In vitro* ADME assay results. (D)Joint clinical score in treated mice. (E) Grip strength test 20 days after dosing, *p＜0.05， **p＜0.01. (F) Serum IL-6 levels significantly reduced in VAV1 MGD group. **P＜0.01. (G). Oral VAV1 MGD dosing induces VAV1 degradation (day 21).

b) Off-target profiling by Proteomics and ICE 90 Safety panel



- High Sensitivity (single cell)
- High Resolution (80000)
- In-depth Coverage >=5000 genes
- All Functional assay
- Dose responsive curve
- 90-target: 4 weeks
- Gold standard for Ion Chanel
- Precise and Fast
- 15-year experience

Figure 6. VAV1 MGD selectivity analysis using DIA based proteomics(A), Off-target and cardiac saftety evaluation by 90 Safety panel(B) and hERG(C) assay.

TPD Discovery with ICE: Broad Assays, Reliable Data

Table 1. Summary of ICE VAV1 *in vitro* assays and data comparison for a selected MGD

Binary/Ternary Complex Assay		ICE data (IC50 or Kd, nM)				Patent Data	
HTRF biochemical assay	Binary	1217				670	
	Ternary	hVAV1	hVAV2	hVAV3		hVAV1	
		18.35	>10,000	>10,000		11	
		mouse/rat	dog	cyno		/	
		18.04/14.28	14.08	17.89		/	
Spectral shift biophysical assay		Binary: Kd=511 Ternary: Kd=16				/	
Cellular NanoBRET assay		IC50=18.45				/	
Jurkat by HiBiT		8.98				7	
JESS analysis		Jurkat	T cell	B cell	PBMC	T cell	B cell
		3.80	0.67	0.96	0.99	0.4	0.7
T cells	Proliferation (Flow cytometry)	0.26				~ 0.3	
	CD69 detection (Flow cytometry)	0.32				~ 0.3	
	IL-2 secretion	0.45				~ 0.3	
B cells	CD69 detection (Flow cytometry)	0.43				~ 0.7	
	IL-6 secretion	0.57				~ 0.8	
	IgG release	4.34				2-3	
TH17 cell	IL-17A secretion by ELISA	1.95				0.3-3	

Summary

ICE's integrated TPD platform, featuring SpS-based screening showcased by CRBN FBS, accelerates progression from hit discovery to *in vivo* validation. Backed by a broad suite of well-established assays — with VAV1 and STAT6 as examples — and customizable modules, it delivers robust activity and safety data to advance your pipeline.