Fully Using the DIANTHUS Technology Platform in Induced Proximity Fields

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Abstract Number:

Introduction

The characterization of biomolecular interactions is fundamental to drug discovery, particularly for complex modalities such as small molecules, molecular glues(MG), and PROTACs. This study presents a comparative analysis of several key biophysical technologies, with a focus on the innovative DIANTHUS platform.

DIANTHUS combines Spectral Shift-based detection with Time-Resolved Immuno-FRET (TRIC) to deliver superior performance in homogeneous assays. Compared to established techniques like HTRF and SPR, DIANTHUS demonstrates exceptional sensitivity, a broad dynamic range, and remarkable resilience to compound interference. These attributes make it an ideal solution for challenging applications, including the high-throughput screening and precise mechanistic study of molecular glues and PROTACs, which function by inducing or stabilizing protein-protein interactions. Our findings position the DIANTHUS platform as a powerful and versatile tool for accelerating the development of next-generation therapeutic agents.

Comparison of Drug Affinity Assay Formats

Assay Formats	Key Advantages	Typical Applications
SpS	Performed in solution, no immobilization Extremely low sample consumption Fluorescent dye	High-throughput
FP/FI	Homogeneous assay (in solution) high throughput fluorescent labeling	High-throughput Competitive binding assays
SPR	Real-time,label-free kinetics high sensitivity, low sample consumption, medium throughput	Kinetic, Affinity

Method

The fluorescence emission is highly sensitive to changes in its immediate environment, which can be modulated by ligand proximity, conformational dynamics of the protein, and variations in local hydrophobicity and charge.

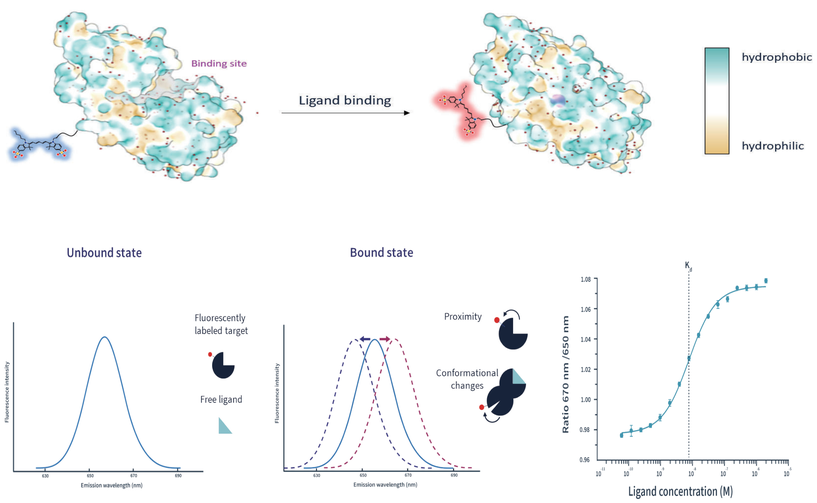
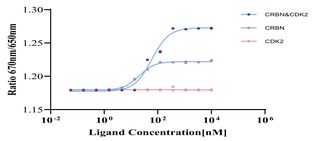


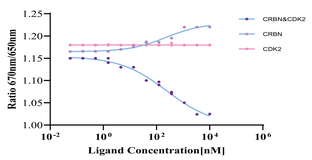
Figure 1: Binding towards a labeled target induces environmental changes to the fluorophore

Case Study 1: HTRF and SpS assay comparison_ CDK2 MG Screening

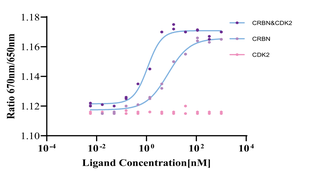
α<1: MG1: α= CRBN Binary/CRBN&NEK7 Ternary =21.8/56.4=0.39

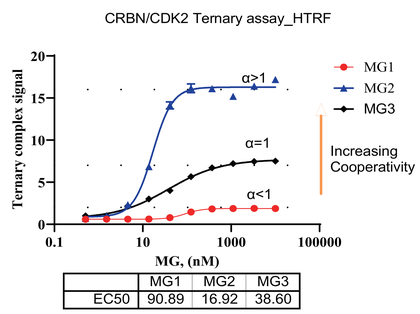


α=1: MG3: α= CRBN Binary/CRBN&NEK7 Ternary =272/216≈1



α>1: MG2: α= CRBN Binary/CRBN& NEK7 Ternary =15.8/2.36=6.7

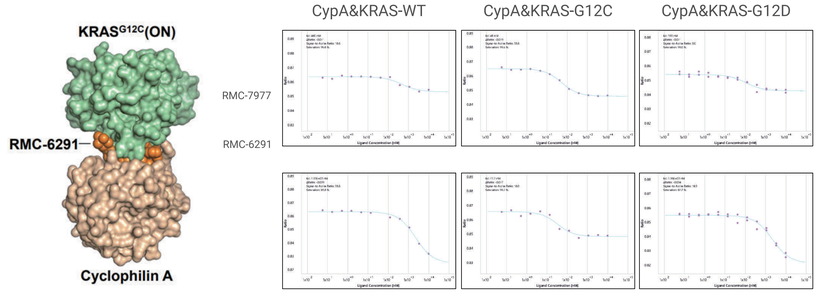




	MG1	MG2	MG3
EC50	90.89	16.92	38.60

Compound	Ternary Binding [nM], HTRF	Binary Kd [nM], SpS	Ternary Kd [nM], SpS	α
MG1	90.89	22	56	0.39
MG2	16.92	16	2.4	6.7
MG3	38.60	272	216	1

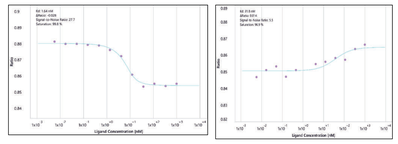
Case Study 2: CypA & KRAS Mutations & MG Ternary Binding Affinity



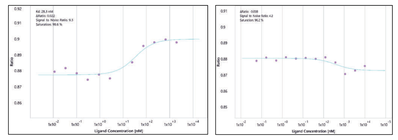
Kd,nM	Targets	Binding _ SPS	Binding _ SPR	HTRF_IC50
RMC-7977	CypA&KRAS-WT	460nM	NA	16.79nM
	CypA&KRAS-G12C	48 nM	23.9nM	NA
	CypA&KRAS-G12D	103nM	256nM	14.7nM
RMC-6291	CypA&KRAS-WT	2130nM	/	5674nM
	CypA&KRAS-G12C	27.7nM	<0.01nM	19.08nM
	CypA&KRAS-G12D	2160nM	/	1433nM

Case Study 3: Library Screening with SpS _ STAT6

PROTAC:

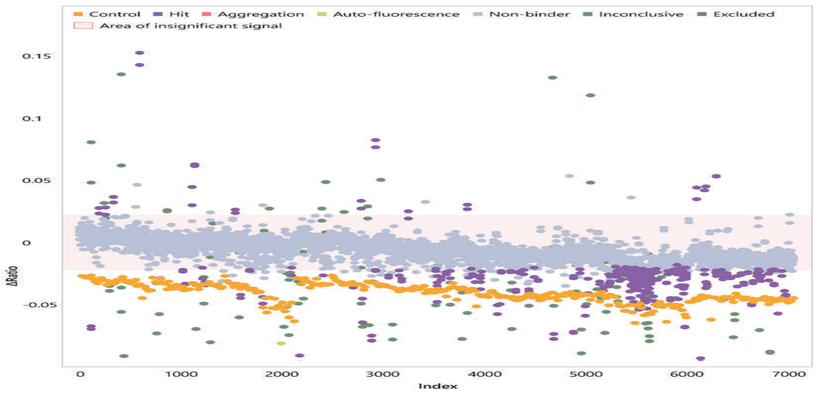


Small molecules:



Compound	SPR,KD(nM)	SPS,KD(nM)
I-2	8.9	1.64
AK-1690	39.4	31.8
STAT6-IN-2	20	28.3
YM-34619	No Binding	No Binding

- STAT6 is an undrugged essential transcription factor in the IL-4 and IL-13 signaling pathways and the central driver of TH2 inflammation in allergic diseases.
- The types of molecules targeting STAT6 are diverse, and their binding mechanisms are not well understood. The lack of clear binding sites makes it challenging to select appropriate screening strategies.
- Biophysical detection of interactions between molecules and proteins is an important means for early screening.



STAT6 Compounds Library Screening by Spectral Shift.

- Positive control signal (orange circles)： I-2(from Kymera KT-621 Patent)
- Hits signal (purple circles)

Summary

- Spectral Shift is a sensitive technology that enabled the rapid identication of hits from small molecules,such as molecular glues, PROTAC in single dose and dose response experiments.
- We established many easy, rapid assays to monitor compound binding by target proteins in solution by utilizing the Echo liquid handler for nanoliter droplet ejection into 384-well plates.
- Spectral Shift method drastically cuts sample consumption and quickly generates robust data, thereby accelerating MedChem analysis for hit finding.