Fragment-Based Drug Discovery (FBDD) Approach for IRAK4

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KEY FINDING

A biophysical approach to finding hits in FBDD

ABSTRACT

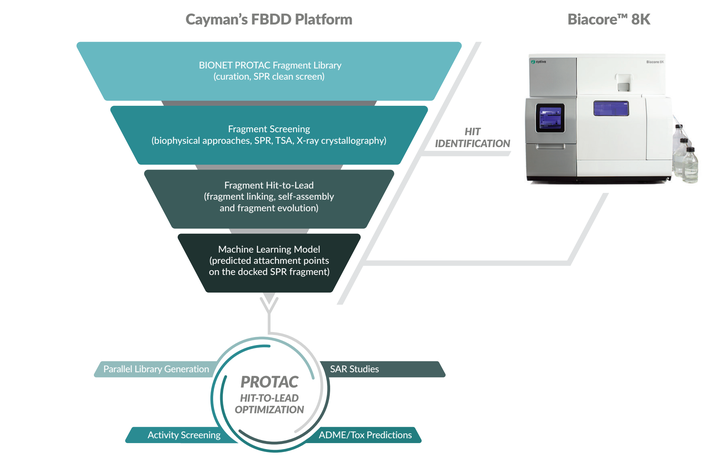
Interleukin-1 receptor-associated kinase 4 (IRAK4) is a serine/threonine kinase that regulates signaling through toll-like receptors (TLRs) and interleukin-1 receptor (IL-1R). Mutations or overexpression of IRAK4 has been implicated in various types of cancers and autoimmune diseases. IRAK4 activity is driven by both its kinase activity and scaffolding function. This necessitates the complete removal of IRAK4 for therapeutic purposes by targeted protein degradation (TPD). In this study, we followed a fragment-based screening approach to provide insight into designing proteolysis-targeting chimera (PROTAC) molecules selective for IRAK4.

We conducted a fragment-based screening study against IRAK4 using our integrated Medicinal & Computational Chemistry Platform. In the first round of the study, a surface plasmon resonance (SPR) "clean screen" of BIONET PROTAC Fragment Library was run to identify and remove fragments that bind non-specifically to the Biacore™ CM5 Sensor Chip. After this initial clean-up step, "binding level screens" were carried out for the 571 remaining fragments to identify binders against the target protein. Top 12 hits from the binding level screens were validated by "affinity screens" to verify binders and estimate affinity (K_D). Machine learning (ML) was then used to design and optimize novel and diverse compounds from the SPR fragment hits which were then used as starting points for PROTAC design. These PROTACs are currently being synthesized and tested.

OBJECTIVE

In collaboration with Key Organics Limited, BIONET PROTAC Fragment Library was selected and screened against IRAK4 using SPR. To perform hit-to-lead optimization on the top fragment hits, Cayman's Medicinal & Computational Chemistry Platform has used machine learning to develop novel and diverse molecules optimized to bind IRAK4 and to serve as starting points for PROTAC design.

WORKFLOW



EXPERIMENTAL SETUP

- IRAK4 (E154-S460) (Cayman Chemical Item No. 42144) was immobilized to a Series S Sensor Chip CM5 (Cytiva) through amine coupling (~15,000 RU).
- SPR experiments were run on a Biacore™ 8K instrument.
- SPR clean and binding screens were run at a single concentration against a blank dextran surface and target surface. For clean screen, fragments were loaded in 384-well PP microplates and screened at 1 mM in PBS-P+ with 2% dimethyl sulfoxide (DMSO). For binding screen, fragments were loaded in 384-well PP microplates and screened at 500 µM in PBS-P+ with 1% DMSO. Solvent correction cycles and positive and negative controls were included.
- Computational modeling was performed for this fragment library against human IRAK4 (PDB ID 8WTF structure) using Schrödinger software.
- Graph neural networks (GNNs) were used to aid in hit-to-lead expansion. The model takes a fragment-docked protein structure as input, converts it into a graph with a set of features, including a user-provided attachment point, and predicts which fragments from a provided library is most suitable to be added at the attachment point.
- The hence obtained lead molecule is docked to IRAK4 again, and the docking score is assessed to see if it is better than that of the hit fragment. If the docking score of the lead is better than the hit fragment, it is considered as a candidate for attachment of the linker and thalidomide head to make the final PROTAC molecule. The designed PROTAC molecule (including the E3 ligase ligand) was subsequently docked to IRAK4.
- PF-06650833 and KT-474 were used as positive controls in both SPR experiments and computational experiments.

RESULTS

SPR Binding Level Screen

To provide deeper insight into the binder characteristics of each fragment, a binding level screen was carried out for fragments at a single concentration of 500 µM (63 fragments were excluded by SPR Clean Screen, not shown).

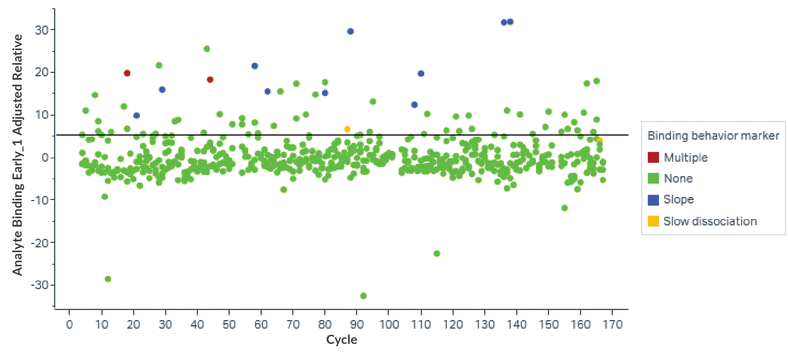


FIGURE 1 – Binding level screen. A total of 571 fragments were screened at a single concentration against dextran surface (reference) and IRAK4 immobilized target surface (active) on a CM5 sensor chip.

SPR Affinity Level Screen and Docking Data for Top Fragment Hits

Top hits from binding level screen were validated by affinity level screens to verify binders and estimate affinity (K_D) (**Figure 2**). To pre-filter and visualize any fragments in the IRAK4 binding site, docking was performed (Glide followed by MM-GBSA for better enrichment) against IRAK4 (PDB ID 8WTF) (**Figure 3**).

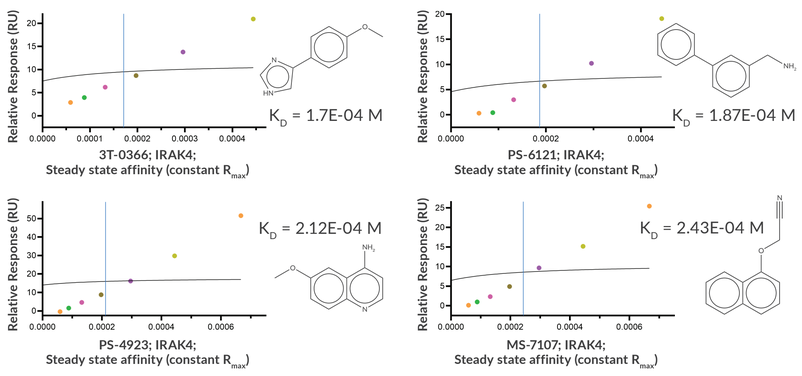


FIGURE 2 – Affinity screen. Steady state affinity was determined for the fragment binding screen hits using an 8-point dose response against a dextran surface (reference) and IRAK4 immobilized target surface (active) on a Cytiva Series S Sensor Chip CM5.

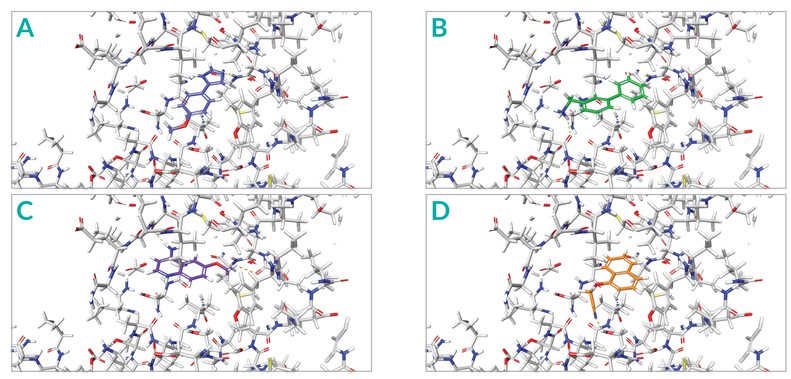


FIGURE 3 – Predicted poses for four of the top scored fragments docked against IRAK4 (PDB ID 8WTF). IRAK4 is colored grey **(A)** 3T-0366 (XP gscore = -7.353) **(B)** PS-6121 (XP gscore = -6.263) **(C)** PS-4923 (XP gscore = -7.113) **(D)** MS-7107 (XP gscore= -7.363).

Hit-to-Lead Computational FBDD

To visualize candidate PROTAC molecules in the IRAK4 binding site, docking was performed (Glide followed by MM-GBSA for better enrichment) against IRAK4 (PDB ID 8WTF).

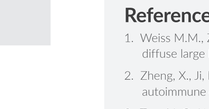
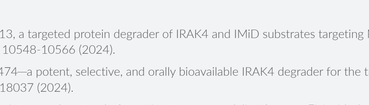
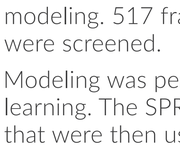
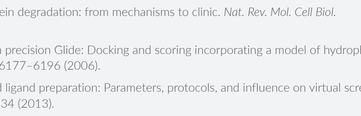
SPR Hit Fragment	Fragment Name	Steady State Affinity (constant R_{max})	SPR Hit Glide Score	Lead Molecule	Lead Molecule Glide Score	PROTAC	PROTAC Glide Score
	3T-0366	1.71E-04	-7.353		-7.448		-10.782
	PS-6121	1.87E-04	-6.263		-9.419		-12.849
	PS-4923	2.12E-04	-7.113		-11.986		-10.393
	MS-7107	2.43E-04	-7.363		-8.966		-10.607

TABLE 1 – Docking results of candidate PROTAC molecules against IRAK4 (PDB ID 8WTF). Molecules were generated from SPR fragment hits. Candidate PROTAC molecules are listed in the table.

Docking Poses for Candidate PROTAC Molecules

FIGURE 4 – Predicted poses for four of the candidate PROTAC molecules docked against IRAK4 (PDB ID 8WTF). IRAK4 is colored grey **(A)** 3T-0366 PROTAC **(B)** PS-6121 PROTAC **(C)** PS-4923 PROTAC **(D)** MS-7107 PROTAC.

SUMMARY

- Our integrated fragment screen workflow was conducted on a Biacore™ 8K instrument against IRAK4, tailored with computational modeling. 517 fragments from BIONET PROTAC Fragment Library were screened.
- Modeling was performed using Schrödinger software and machine learning. The SPR hit fragments were used to create lead molecules that were then used to design candidate PROTACs.

References

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