Integrated solutions provider in drug discovery

Axcelead Drug Discovery Partners, Inc.

AXCELEAD
Drug Discovery Partners

High-Throughput Affinity Selection Mass Spectrometry (HT-ASMS)

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— Discovery of novel small molecule drug candidates —

It takes many years and a great deal of effort to develop a new therapeutic agent. Traditionally, high-throughput screening (HTS) has been used with biochemical assays to identify initial candidate compounds, but this approach takes more time to identify hit compounds and has an increased incidence of false positives.

HT-ASMS is a technique for identifying hit compounds based on biological activity using binding to the target molecules as an indicator, unlike the conventional approach to evaluating compounds based on their responses to the target molecule. HT-ASMS can be used to identify allosteric binders to the target molecule and to search for compounds via target protein degradation (TPD).

HT-ASMS enables the discovery of small molecules that bind specifically to target DNA and RNA. This technology is a novel solution for small molecule drugs, providing any targets and projects with the highest likelihood of success.

Axcelead Drug Discovery Partners (ADDP) has now developed an online LC/TOF-MS system dedicated to HT-ASMS, which combines size-exclusion chromatography and reversed-phase chromatography. In the first step, size-exclusion chromatography is employed to isolate complexes of target molecules and compounds. In the next phase, reverse-phase chromatography is used to separate compounds from the complex for mass analysis with TOF-MS*. This method is called Two-Dimensional Liquid Chromatography-Affinity Selection Mass Spectrometry (TDLC-ASMS), which enables rapid screening of our proprietary library diverse enough to identify compounds that bind to the target molecules.

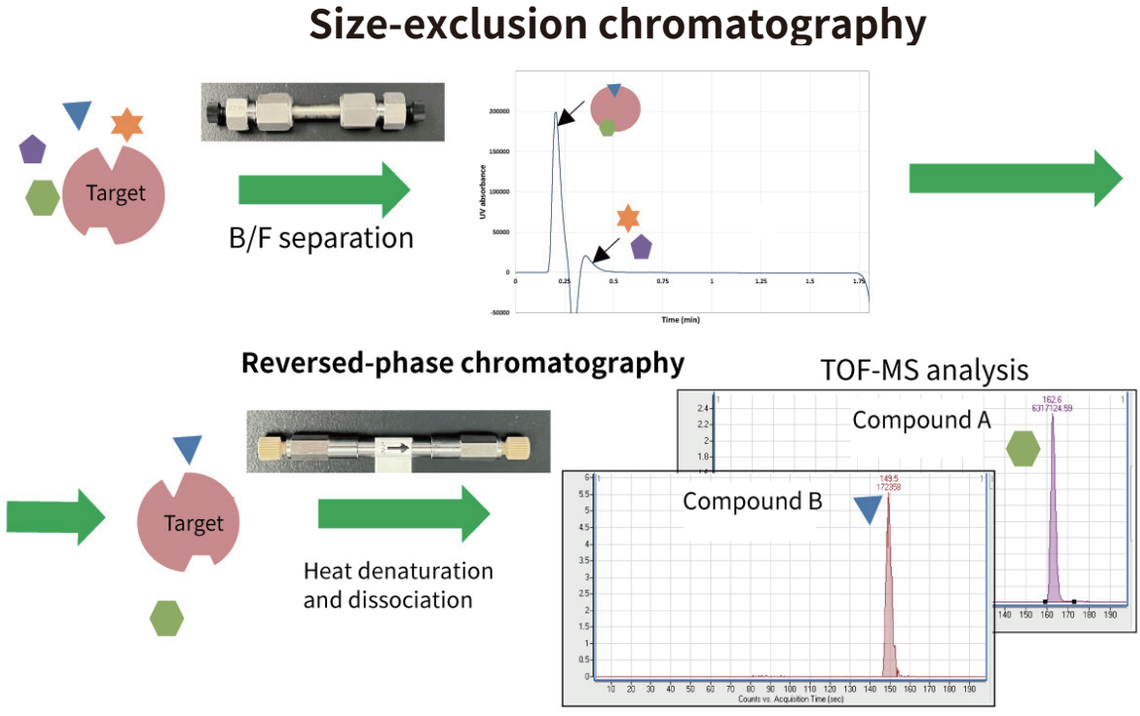
TDLC-ASMS

1. Incubates target molecule and library compounds

2. Separates a target molecule in complex with bound compounds from unbound (free) compounds with size-exclusion chromatography (Bound/Free (B/F) separation)

3. Isolates bound compounds with reversed-phase chromatography

4. Detects and identifies the bound compounds with TOF-MS



Advantages of label-free screening

Conventional approaches require fluorescent labeling of the target molecule or ligands to detect the binding response. The labeling process itself can alter the molecular structure and/or function and may affect activity. The HT-ASMS technique, meanwhile, allows for directly identification of hit compounds without needing to build a biochemical-based assay system. Furthermore, direct measurement in Mass Spectrometry (MS) of compounds bound to target molecules reduces the risk of false positives and other artifacts.

A faster, more efficient, and highly flexible assay system

Even the most cutting-edge technology will be of little practical use in drug discovery if the throughput is too low. We have developed a high-throughput screening system that uses high-resolution TOF-MS and pooled libraries. The system can rapidly identify compounds that bind to the target molecule from our extensive, high-quality library of approximately 320,000 – 500,000 compounds derived from our lead-like small molecule library.

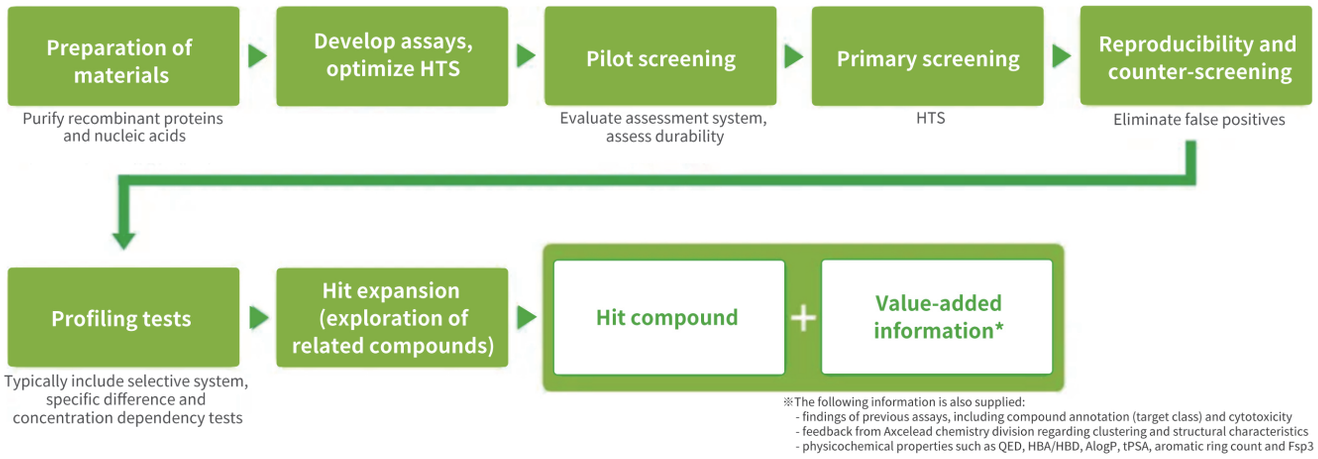
Key benefits of the Axcelead library

Derived from pharmaceutical company to ensure quality

Emphasis on lead-likeness and molecular diversity

Most compounds designed in-house

The quality of materials is another key factor behind the success of HT-ASMS. Axcelead boasts considerable experience and expertise in developing hit compounds by design and preparation of materials such as proteins and nucleic acids. High-quality materials improve the reproducibility of HT-ASMS and make it easier to identify genuine hit compounds.



※The following information is also supplied:
- findings of previous assays, including compound annotation (target class) and cytotoxicity
- feedback from Axcelead chemistry division regarding clustering and structural characteristics
- physicochemical properties such as QED, HBA/HBD, AlogP, tPSA, aromatic ring count and Fsp3

HT-ASMS facilitates the discovery of new targets in drug discovery

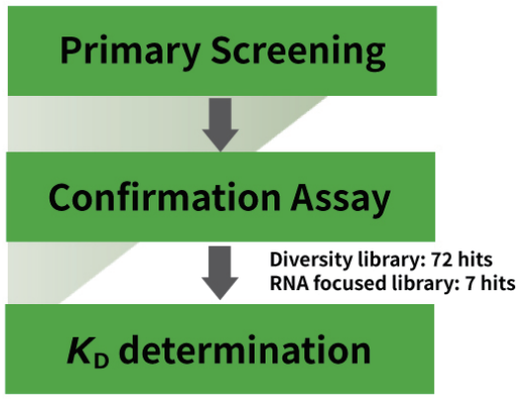
Nucleic acids have traditionally been considered unsuitable targets for small-molecule drug discovery. However, recent studies have reported that some DNA and RNA molecules can adopt tertiary structures and regulate gene expression and splicing functionality upon binding to small-molecule compounds. However, it is more difficult to build biochemical assays to explore compounds that act on nucleic acids where the properties are unknown. HT-ASMS provides a fast and efficient way to obtain compounds that act directly on the target RNA/DNA. Compounds obtained via this promising new technique can lead to breakthrough developments in the new drug discovery domain.

Case study-1: Drug discovery for RNA targets

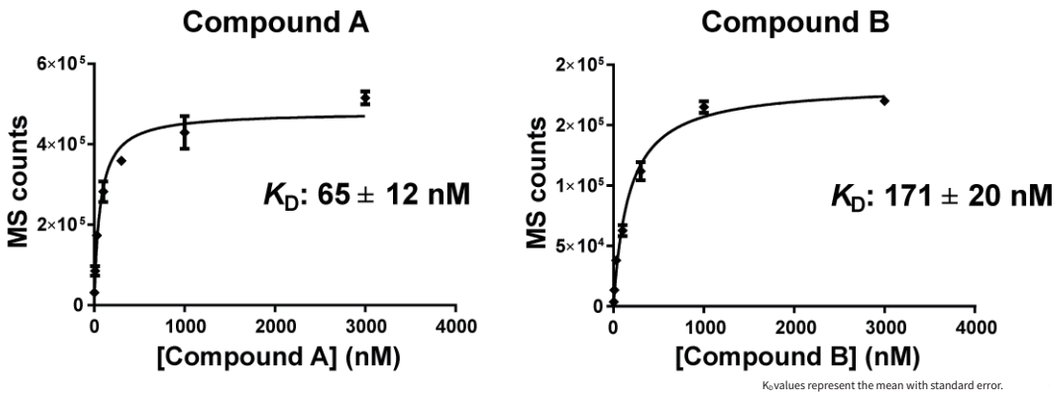
> Exploring compounds that bind to FMN riboswitch with HT-ASMS

Target
FMN riboswitch

Diversity library: 140K compounds
RNA focused library: 6.4K compounds

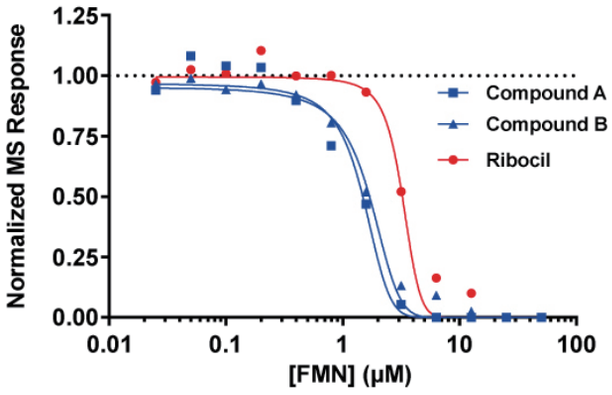


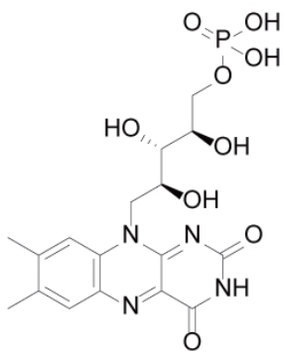
> Quantifying binding affinity of hit compounds with FMN riboswitch based on dissociation constant KD



K_D values represent the mean with standard error.

> Use competitive testing with ligand compounds to predict binding sites and compare binding affinity between compounds





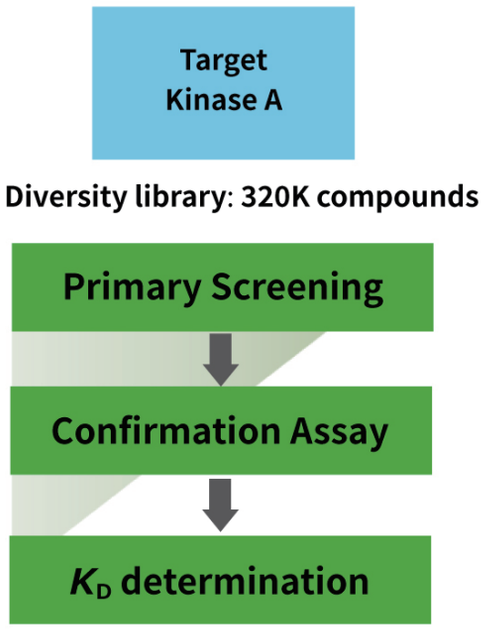
Ligand compound:
Flavin mononucleotide (FMN)

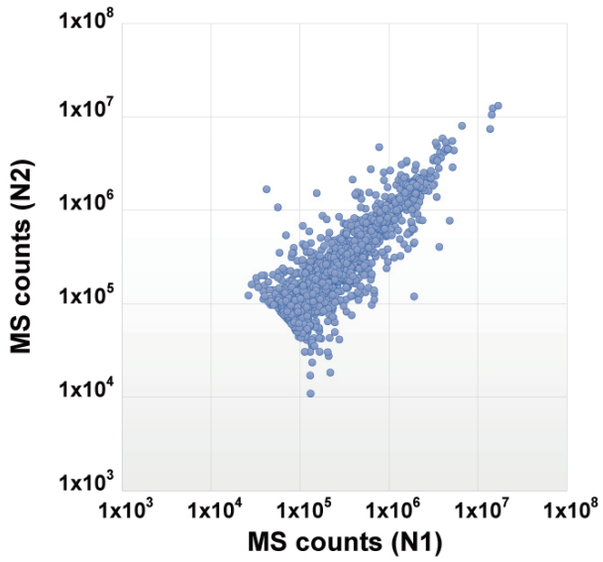
The quest for highly novel compounds

If you take the same approach to compound exploration as your competitors, you might never discover that one truly unique compound. Using HT-ASMS provides you with a much better opportunity for finding unique compounds compared to conventional screening approaches, which typically utilize functional activity. The unique compound that differentiates from your competitors could be the one that leads to a revolutionary new drug—even if it involves a known target molecule.

Case study-2: Small-molecule compound exploration of Kinase A with HT-ASMS

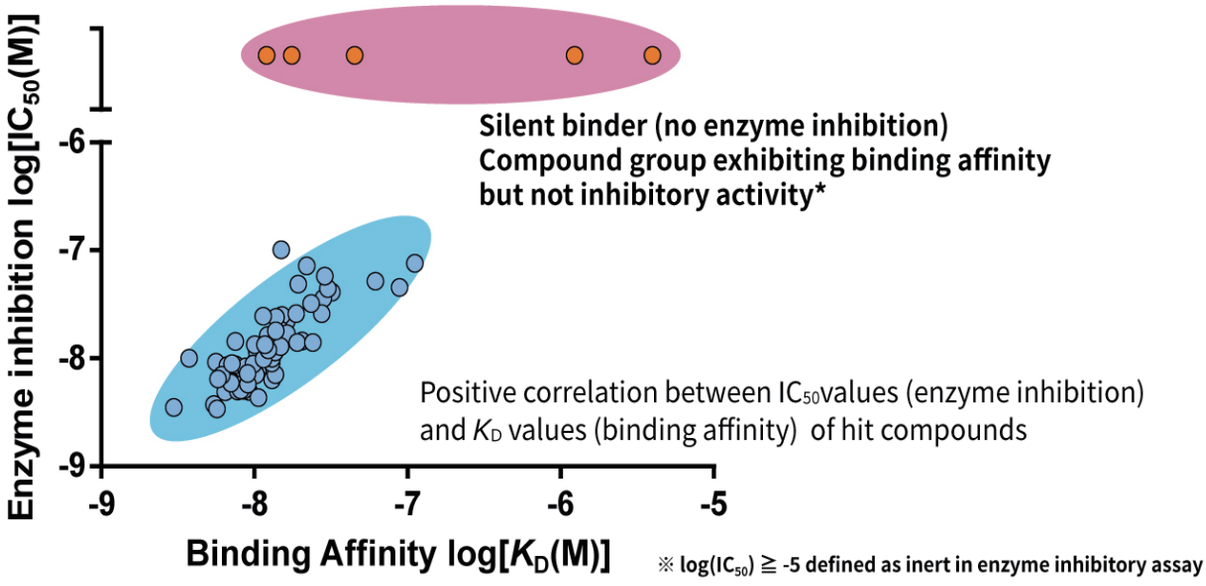
> Explore compounds that bind to Kinase A with HT-ASMS



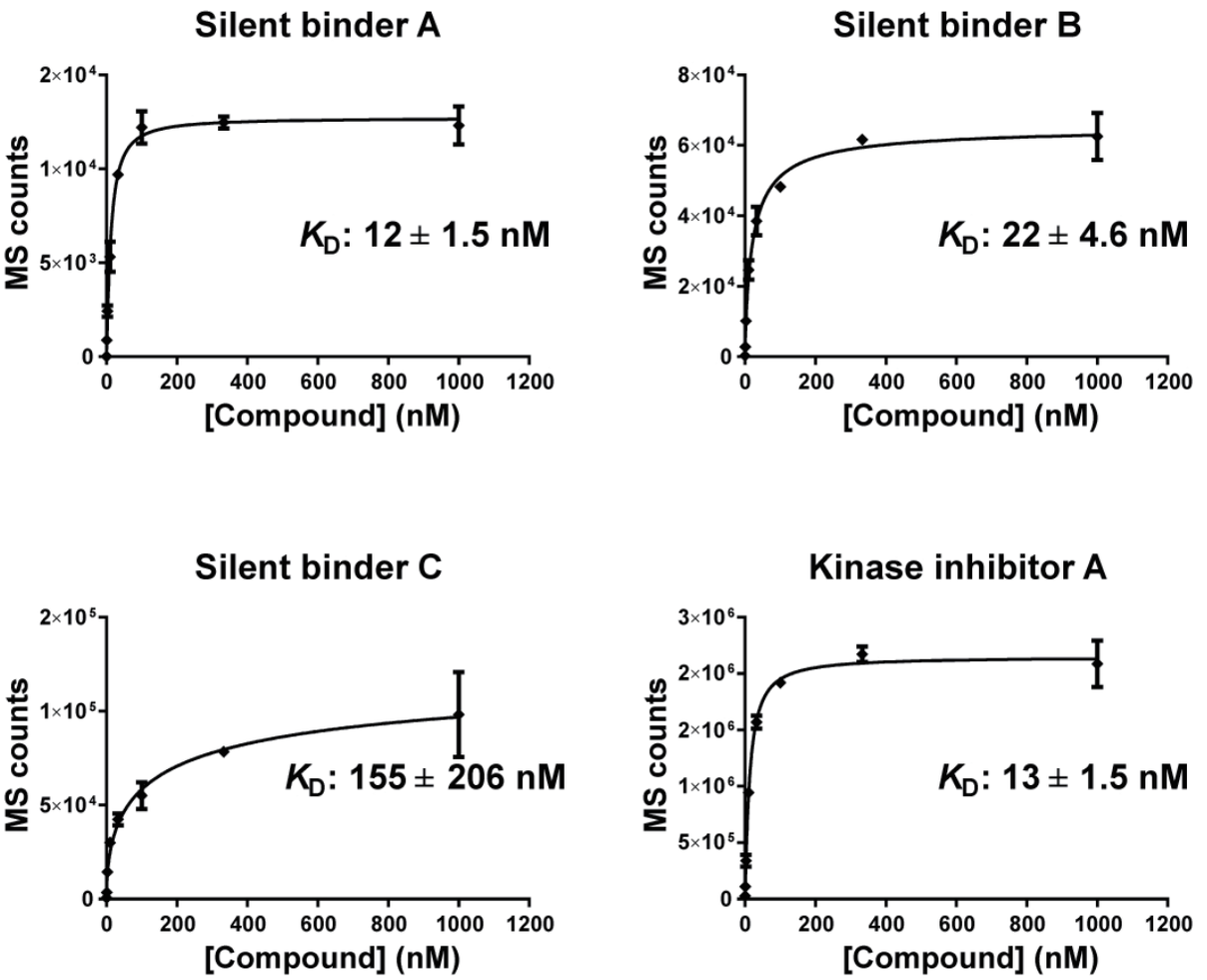


N1 and N2 correlation of the primary screening with HT-ASMS in two-round analysis

> Identify compounds that exhibit with inhibitory activity toward the target molecule, as well as so-called silent binders that do not exhibit inhibitory activity. Silent binders can potentially be used as ligands in target protein degradation.



> To determine the KD value of a silent binder enables to quantify binding affinity for the target molecule.



K_D values represent the mean with standard error.

For hit compounds identified via HT-ASMS, Axcelead assists lead optimization through hit expansion and various profiling techniques.

Hit expansion through the utilization of 1.5 million compound library

Further evaluation using both phenotypic and biochemical assays

Biophysical assay and crystal structure analysis

Axcelead can assist you to create a pipeline for new drug discoveries in the rapidly advancing field of small-molecule drugs.

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