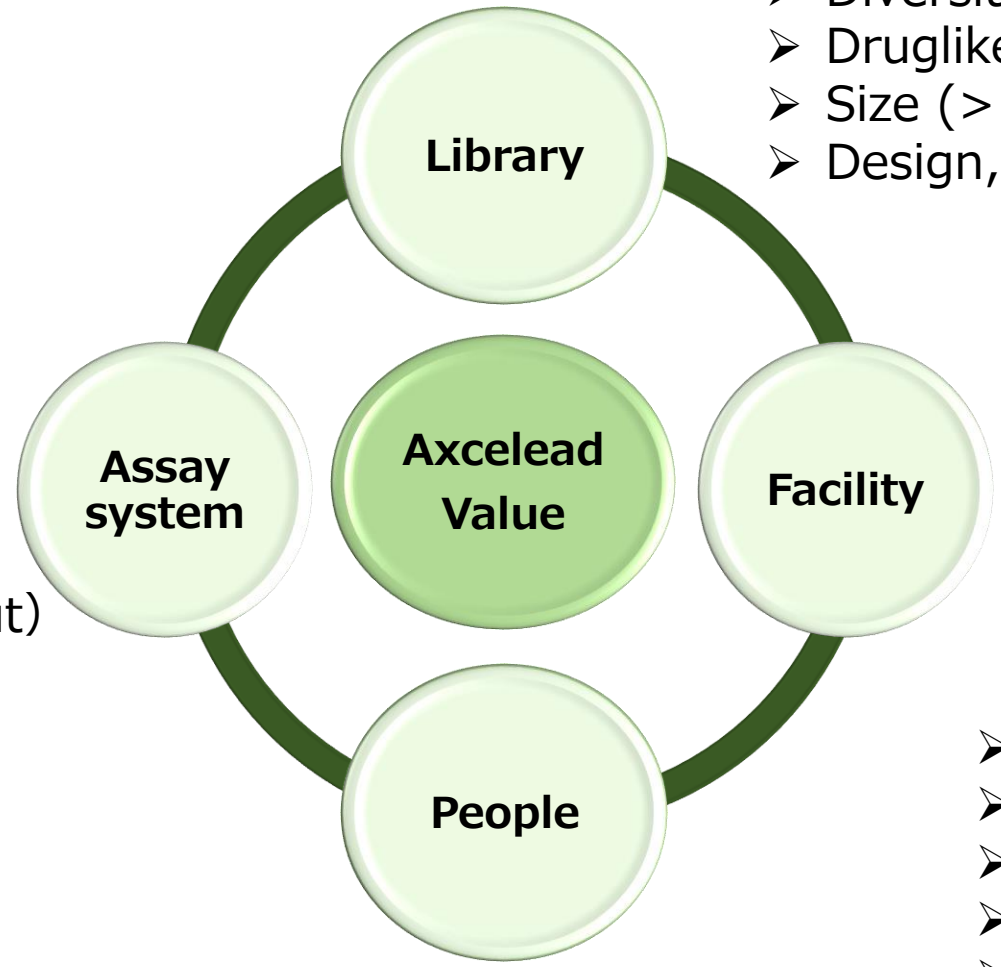
Integrated HTS platform for innovative drug discovery

Axcelead Drug Discovery Partners Inc.
Discovery Science
Discovery Biology

AXCELEAD
Drug Discovery Partners

Key Factors for Hit Identification

- Diversity
- Druglikeness
- Size (>1.0 million)
- Design, Synthesis, Purchase



- Quality, Speed, Cost
- Throughput
- Robustness
- Disease relevancy
- Phenotypic rule of three (Cell X Stimulant X Readout)
- Profiling
- Data analysis

- Robotics
- Plate readers
- Dispensers
- Systems
- Maintenance

- Knowledge, Skill, Experience
- Expertise
- Strategy planning
- Problem solving ability
- Reliability
- Communication
- Collaboration

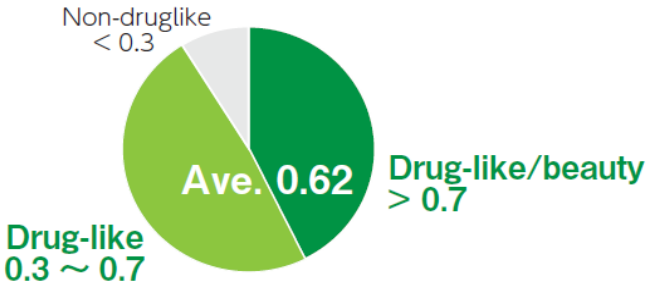
- ***Axcelead provides comprehensive hit identification services through our integrated platform***

Axcelead Compound Library

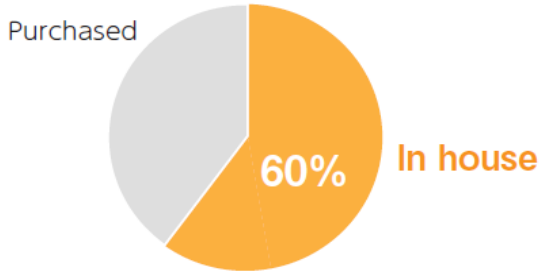
>1,500,000 Compounds

Quality

Lead likeness
QED: Quantitative Estimate of Drug-likeness



In-house compounds



Library sets for HTS

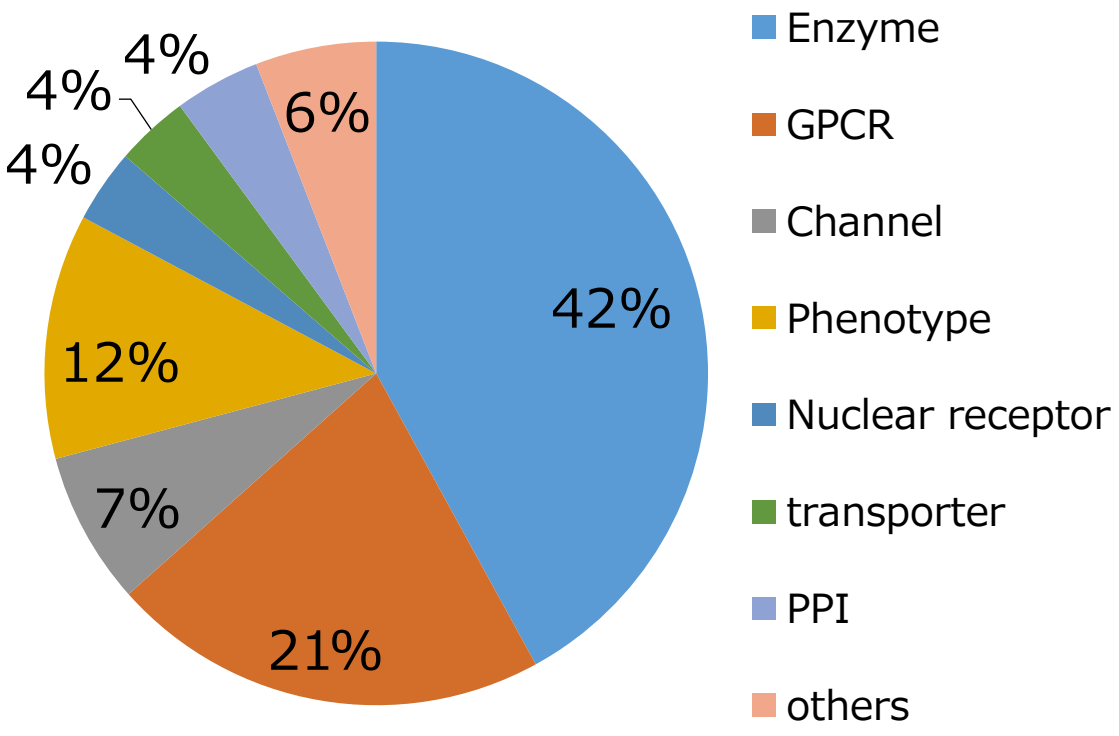
■ **Diversity libraries**
- Single library 126,000 compounds
- Pooled library 500,000 compounds (Standard 320,000 cpds)

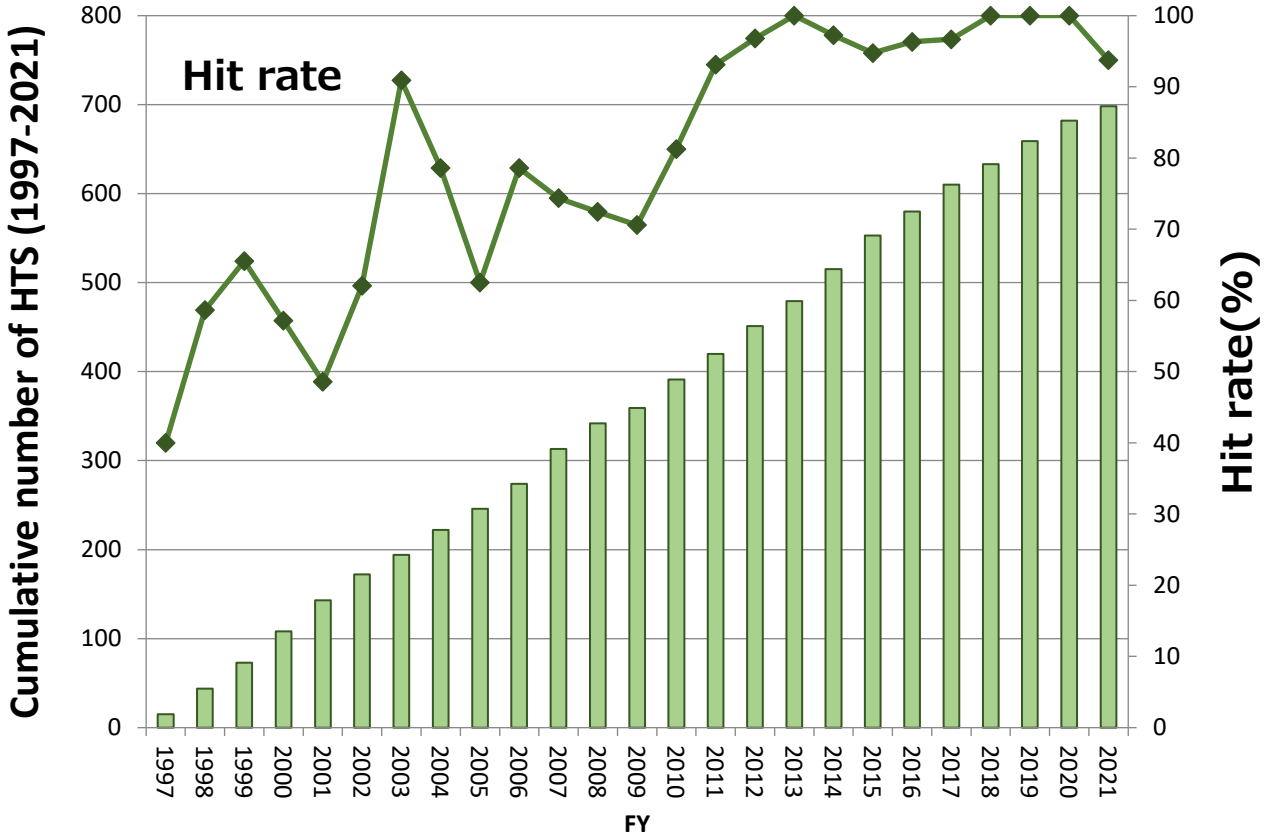
■ **Focused libraries 41,000 compounds**
- Libraries for target classes (Kinase, GPCR, Protease, PPI, etc.)
- Macrocyclic
- RNA
- Covalent
- Extended rule of 5
- Natural product
- Annotation

▶ Biologically annotated library is available for phenotypic screening
▶ We are also able to construct a focused library selected from 1.5 million compounds library by virtual screen

Track Record of HTS Campaigns

Target class





- ***We have successfully achieved around 700 HTS campaigns for various target classes with high hit rates***

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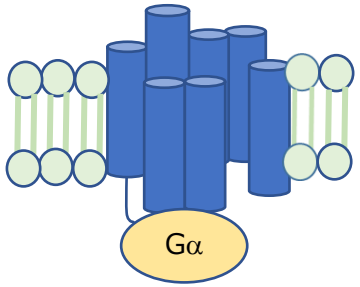
Assay Platforms

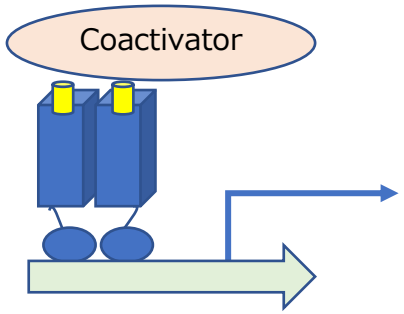
GPCR

- cAMP assay
- Ca^{2+} flux assay
- Reporter gene assay
- Arrestin/Internalization assays
- Binding assay
- Impedance assay



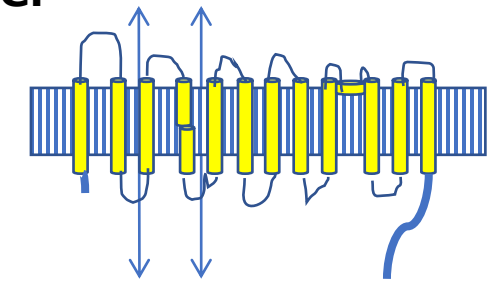
Nuclear receptor

- Binding assay
- Cofactor recruitment assay
- Reporter gene assay
- Nuclear translocation assay



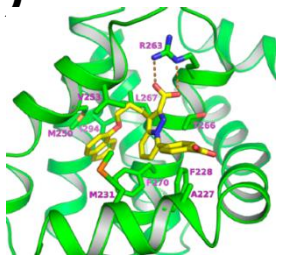
Ion channel / Transporter

- Ion influx assay
- Membrane potential
- Electrophysiology
- Substrate uptake
- Binding



PPI (protein-protein interaction)

- TR-FRET/Alpha screen assay
- ELISA
- NanoBit/BRET
- Two-hybrid assay
- Biophysical assay



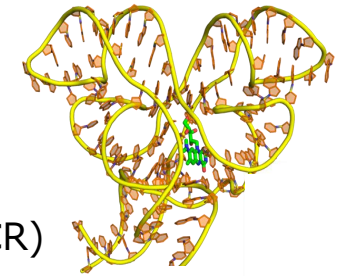
J Med Chem **56** 9635–45 (2013)

Enzyme

- Luminescence, Absorbance, Fluorescence, TR-FRET
- Alphascreen, ELISA
- Radiometric assay
- Label-free assay (e.g. Rapidfire-MS)
- Coupling assay
- Global kinase panel

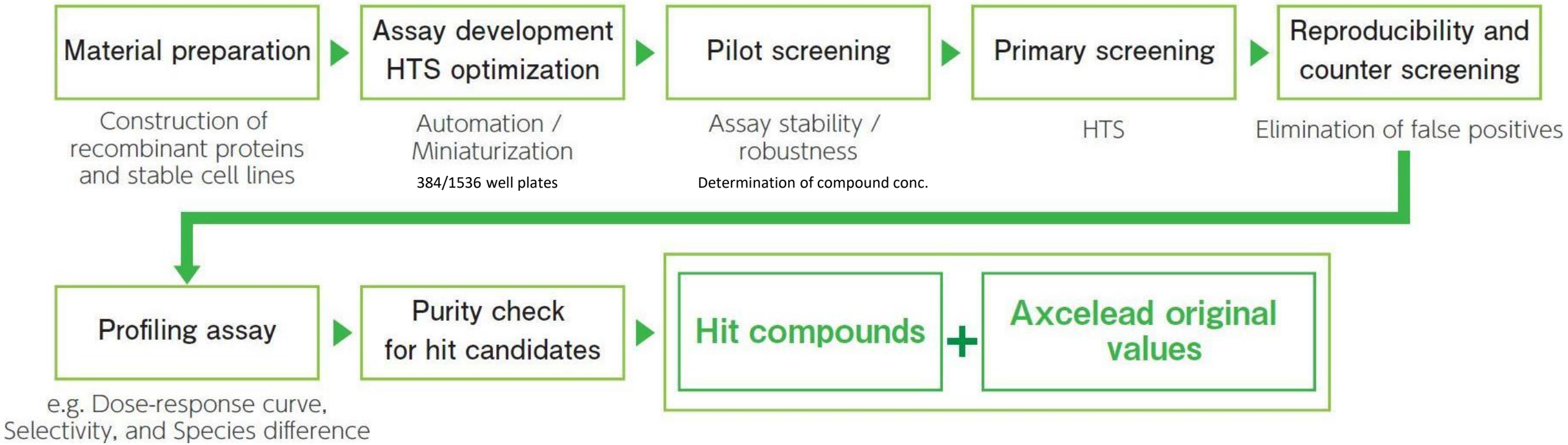
Nucleic acid

- Biophysical assay (e.g. ASMS)
- Fluorescence probe binding
- FRET
- Cell-based assay (Reporter gene, RT-qPCR)



General Hit Finding Process

■ Axcelead comprehensive screening services



We can also conduct HTS campaign using client's assay system

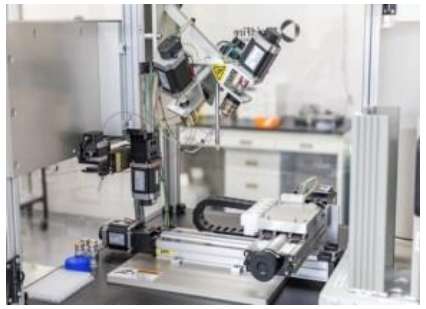
Facilities

- Ultra High Throughput Screening capabilities
- State-of-the-art equipment compatible with diverse assays
  - Acoustic liquid handling system (Echo)
  - Biochemical screening (Envision, SpectraMax)
  - Calcium/Thallium flux screening (FDSS, FLIPR)
  - High-content screening (Incell6000)
  - Live cell imaging (Incucyte)
  - qRT-PCR (QuantiStudio)
  - HT-Mass spectrometry screening (Rapidfire-MS, Tof-MS)
  - Electrophysiological screen with HT-autopatch systems (SyncroPatch)
- Data analysis platform to support all screening processes
- Ability to perform HTS under BSL2 conditions (iPSC,Primary cells,Lentivirus etc.)
- Ability to perform Radioactive HTS

- Fully automated screening system (FUJIFILM Wako)

- Acoustic droplet dispensing syster.. (Labcyte)

- Syncropatch 384
- (Nanion)



- RapidFire (Agilent)

- QuantiStudio 12K Flex Realtime PCR (ThermoFisher)

- IN Cell Analyzer 6000 (Cytiva)

HTS Cascade

Pilot screen (n1, 2dose)

↓

Primary screen (n1, 1dose)

↓

Counter assay (To exclude false positives)

↓

Clustering
Dose response test (IC_{50}/EC_{50}) (n2, 6 dose, Ave.320 cpds)
Purity check of compounds

↓

Hit compounds

Hit finding

Hit report → Chemical structures for up to 320 hit compounds are disclosed

↓

Hit expansion

- Evaluation of related compounds
- Parallel synthesis with HT-chemistry
- SAR analysis

Profiling

- Selectivity/Species difference
- Mode of inhibition/activation
- in vitro ADME-tox assay
- Cellular assays

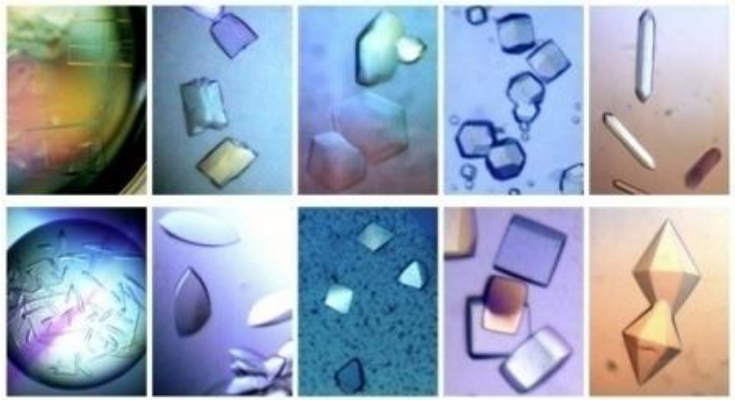
Post HTS services

↓

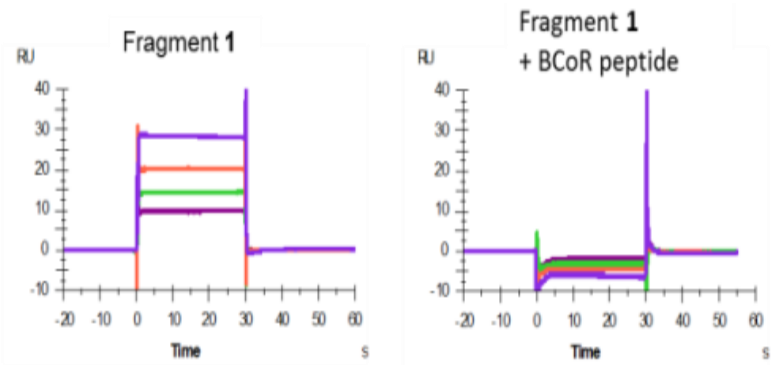
Advanced hit

In vitro Assay Platform for Profiling

- Biochemical assay (Potency/Selectivity/Species difference for SAR)
- Mode of action/ Kinetics analysis and Profiling assay
- Cell-based assay (Cellular target engagement, Cellular function etc.)
- Biophysical analysis for target-compound interaction assay
  - AS-MS, TSA, NMR, ITC, SPR, X-ray crystallography

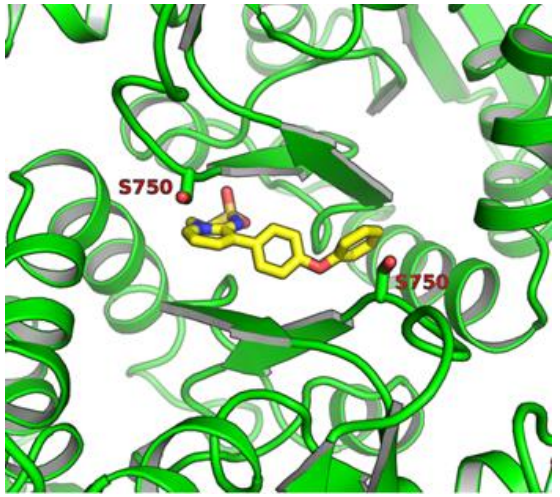


Competition experiment



Kinetics assay with SPR

Thermodynamics assay with ITC



X-ray crystallography

Neuropsychopharm **44** 961–70 (2019)

- ***We can drive drug discovery by using various technologies led by multidisciplinary teams***

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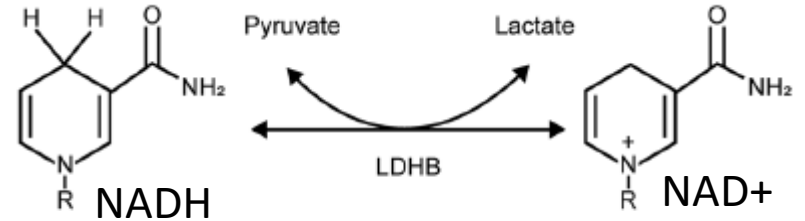
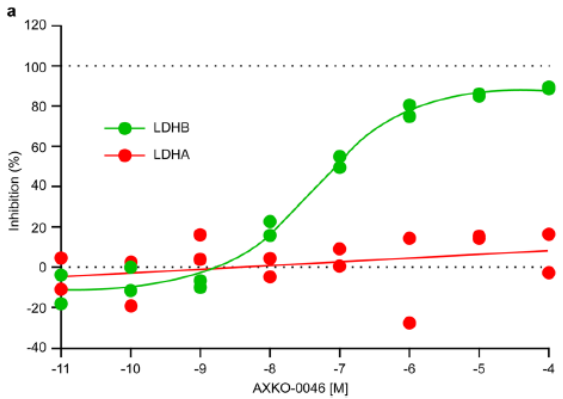
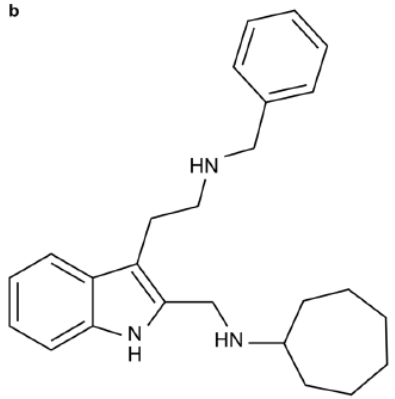
Discovery of Enzyme Inhibitors using Rapidfire-MS

LDHB







HTS cascade

Primary screening (ca. 370,000 compounds)

- Diversity pooled library 10 μM, N=1
- Enzyme assay with Rapidfire-MS

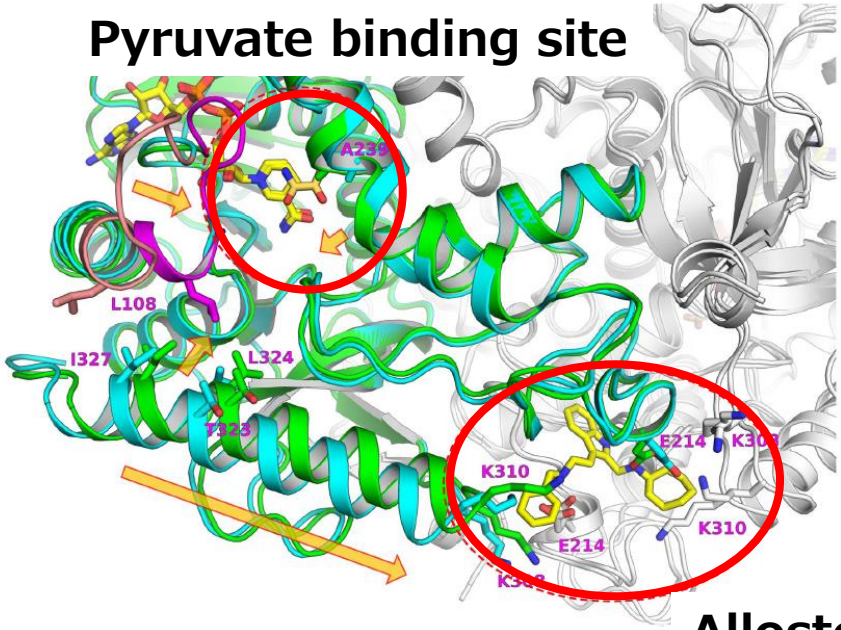
Deconvolution assay (ca. 800 cpds)

- Positive compounds from primary screening 30 μM, N=1

Dose response test

- 4-5 dose, N=2
- Selectivity test (LDHB/LDHA)
- Clustering
- Purity check
- Evaluation of related compounds

Hit compounds



Sci.Rep 2021 11:21353

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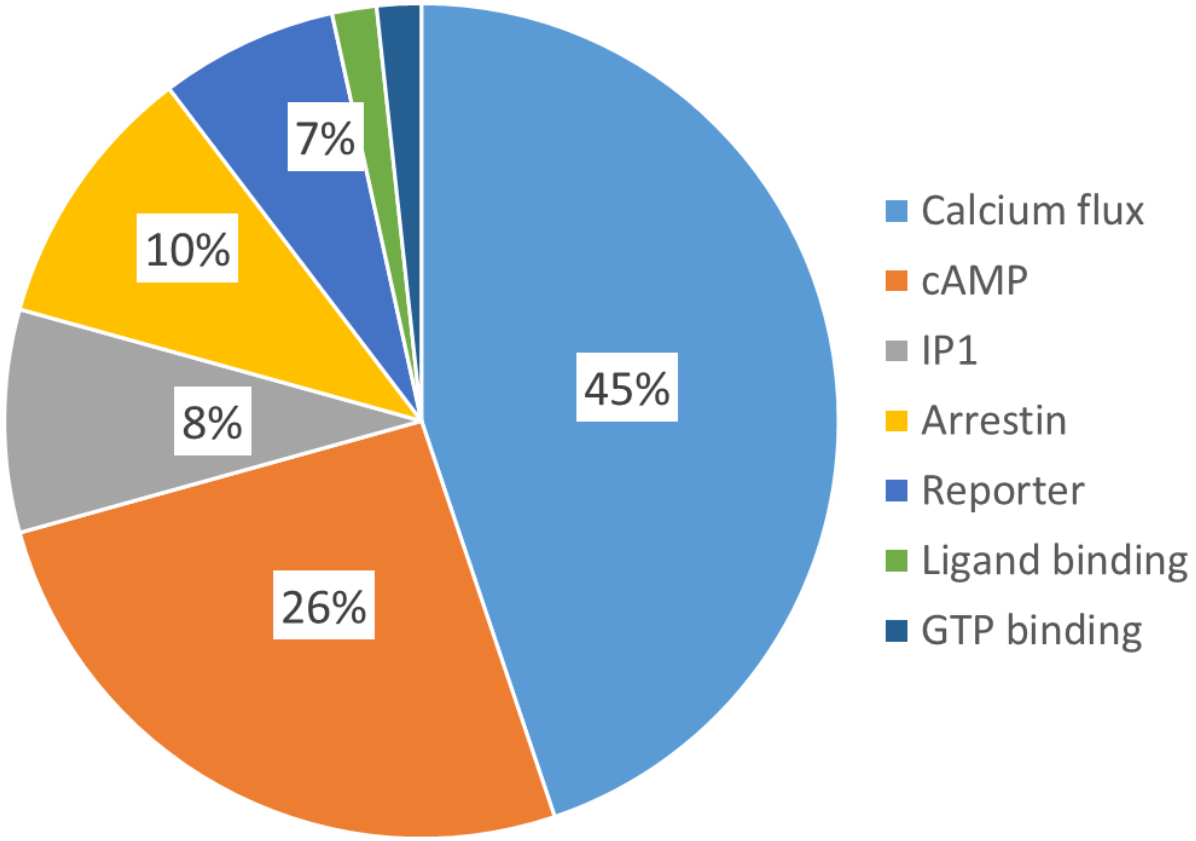
Discovery of GPCR Biased Ligands

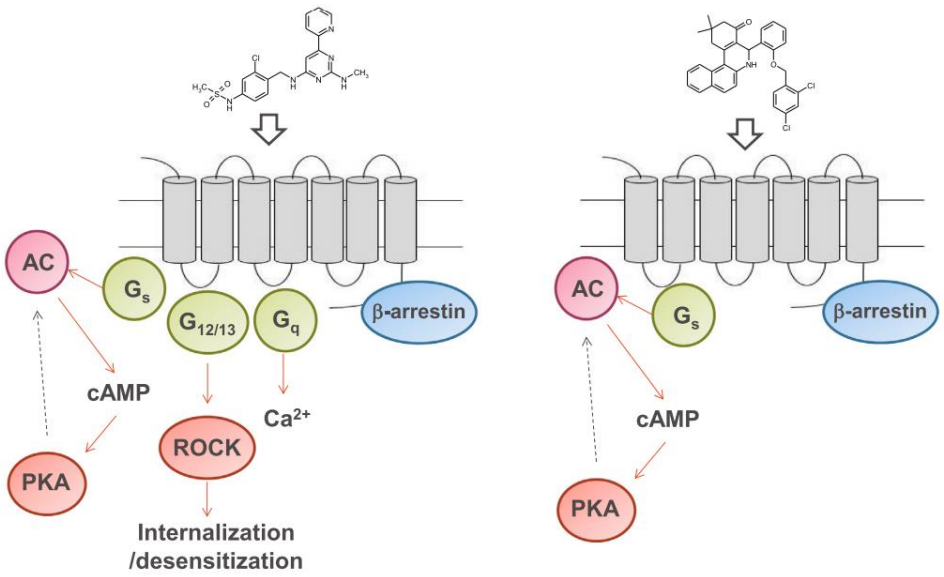
Track record of primary assays in HTS campaign targeting GPCRs (2011-2019)



GPR39 positive allosteric modulators

Library: >600,000 cpds at 3μM
Primary assay: FRET (cAMP), PAM mode

Gs biased



Biochemical Pharmacology 140 (2017) 105–114

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Discovery of RNA Binders using HT-ASMS

High-throughput Affinity Selection Mass Spectrometry for FMN riboswitch RNA binders

Diversity Library: 140,633 cpds
RNA focused Library: 6,400 cpds

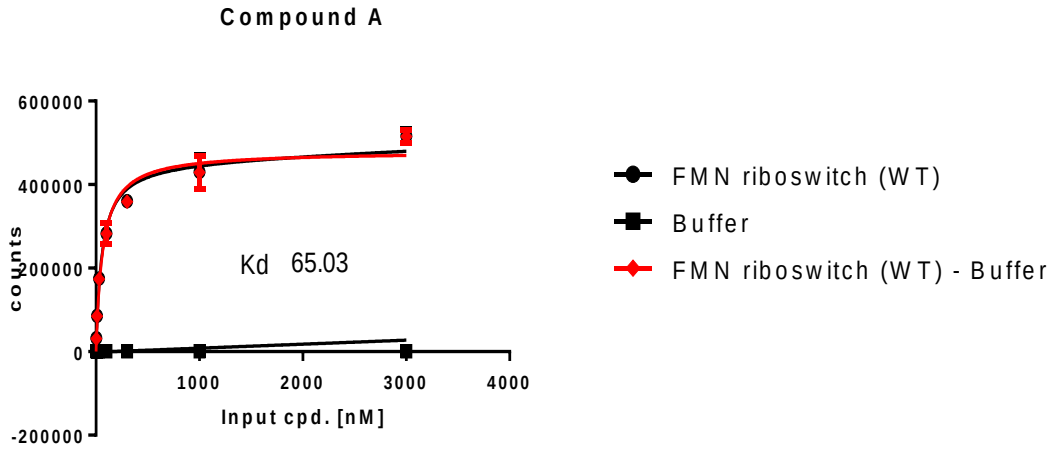
FMN riboswitch RNA 5 μM
Compound conc.: each 0.5 μM
Assay format: 400 cpds/pool

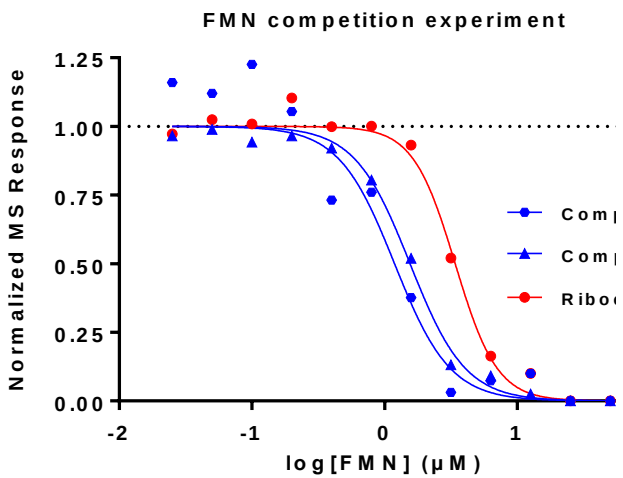
Reproducibility test

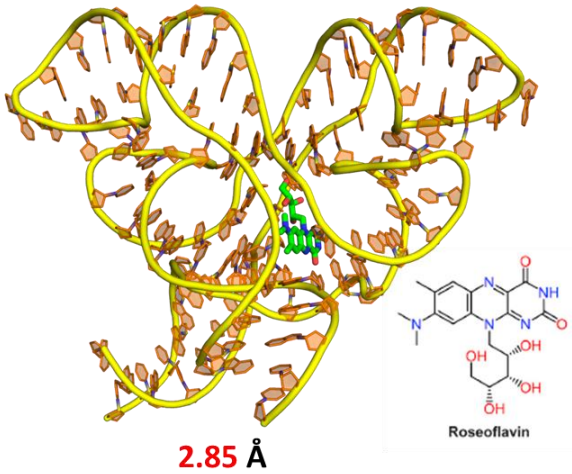
Counter Screen using scramble RNA

Confirmation assay
Diversity Library : 72 hits
RNA focused Library : 7 hits

Kd determination







In house data

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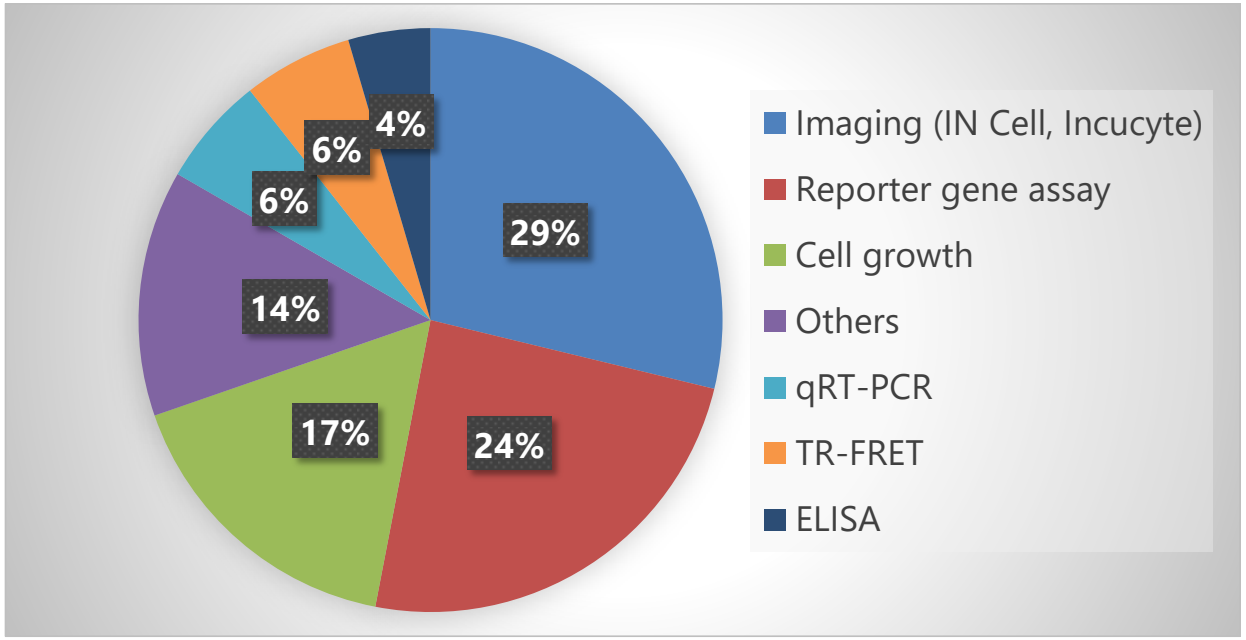
Track Record of Phenotypic Screening

Track record for >70 PDD programs

Assay methods



Assay methods	Usual screening library size
Imaging	100K compounds
Reporter gene assay	400K compounds
Cell growth Incl. synthetic lethality	400K compounds 3200 compounds
qRT-PCR	30 K compounds
TR-FRET	100K compounds
ELISA	100K compounds
POI-HiBit screening (for degrader screening)	100K compounds

➢ ***We can propose the best screening strategies according to your needs***

Phenotypic Screening using iPS Cells

Aβ uptake assay in iCell® TREM2 mutant Microglia

HTS cascade

Primary screening (c.a. 4000 compounds)

- Biologically annotated compounds, > 3,000 cpds
- 3 μM, N=1
- Phagocytosis assay and cytotoxicity (CellTiter-Glo® Luminescent Cell Viability Assay)

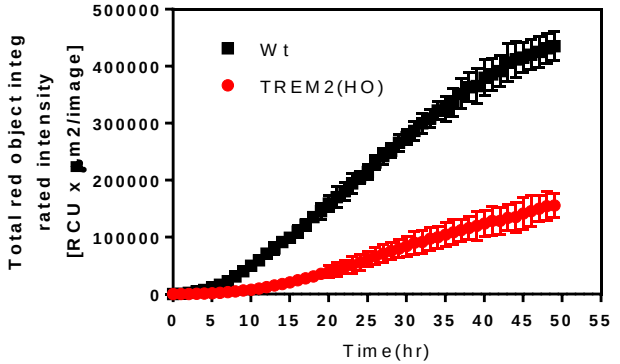
Reproducibility test (350 compounds)

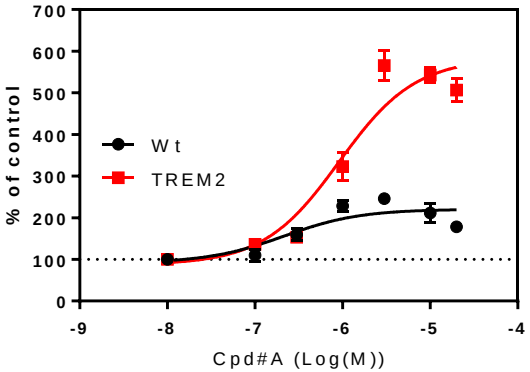
- Positive compounds from primary screening
- 3 μM, N=1
- Phagocytosis assay and cytotoxicity (CellTiter-Glo® Luminescent Cell Viability Assay)

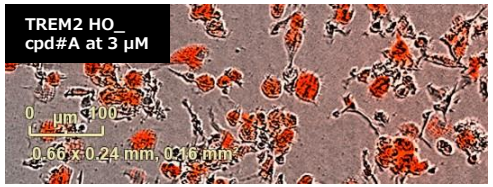
Dose response test (24 compounds)

- Selected compounds from reproducibility test
- 6 dose, N=2
- TREM2 mutant and WT
- Phagocytosis assay and cytotoxicity (CellTiter-Glo® Luminescent Cell Viability Assay)

Hit compounds







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Combination Screening Using Annotation Library

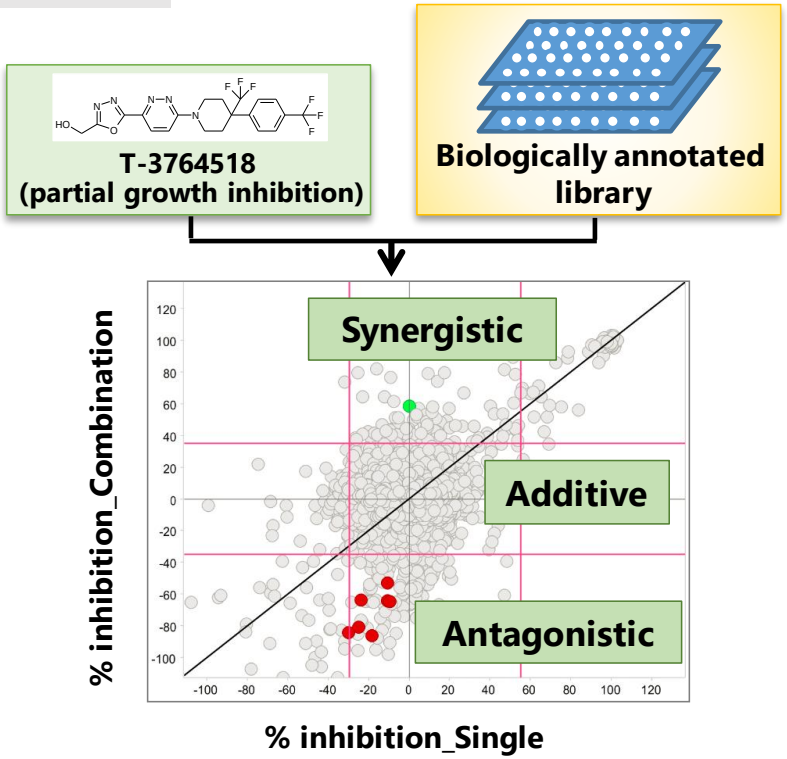
- ➢ Combination screening for discovery of a synthetic lethal compound or MOA analysis
- ● Your interested compounds x Annotation library

  ⇒ Search for combination partners which act as synergistic or antagonistic.

Example

Elucidation of resistant mechanism for cancer cells against SCD1 inhibitor.

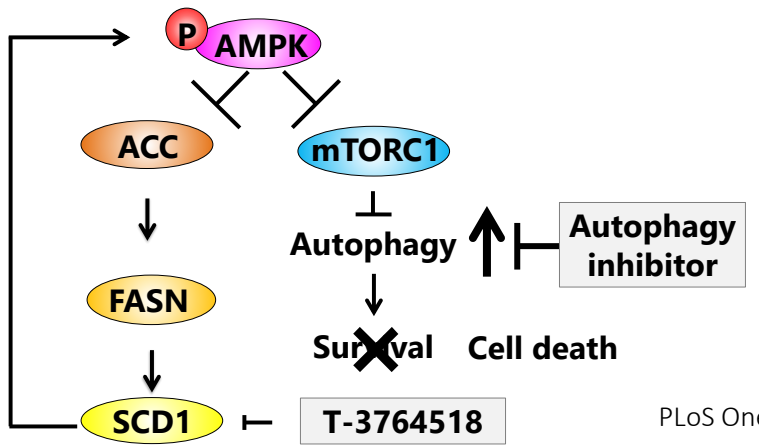
<Strategy>



<New finding>

Feedback activation of autophagy is a key resistance mechanism against SCD1 inhibitor-induced cell growth inhibition.

SCD1 inhibitor and autophagy inhibitor co-treatment



PLoS One. 2017 Jul 13;12(7):e0181243.

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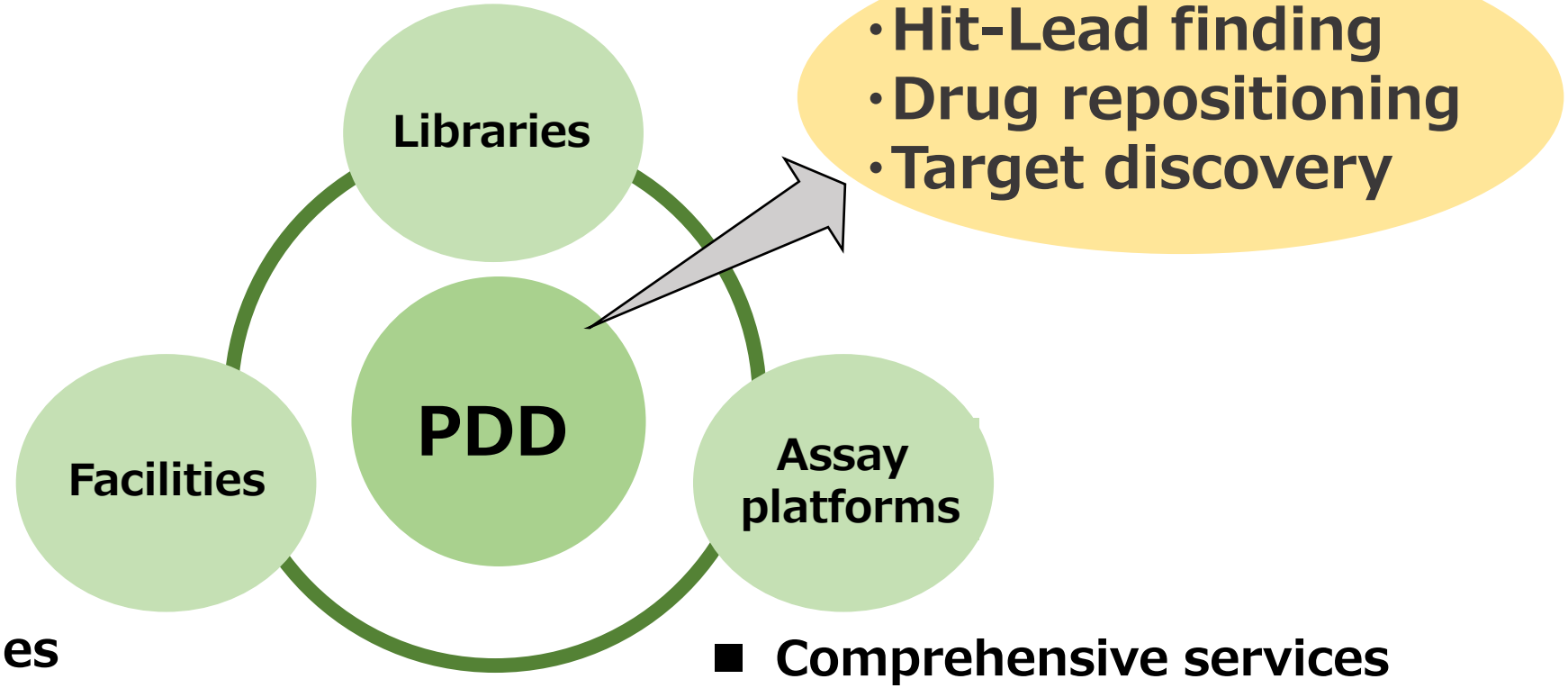
Phenotypic Drug Discovery (PDD) in Axcelead

■ High quality and attractive libraries

・Diversity
・Biological annotation
・FDA approved
・Focused



■ Cutting edge facilities

・Automation system
・Wide rage of devices
  Envision
  Incell6000
  qRT-PCR system etc.
・BSL2 laboratory

■ Comprehensive services

・Cell construction
・Assay platforms
・Target discovery with Crispr Cas KO screen
・MOA analysis
・Biochemistry and Biophysics
・Target deconvolution

Integrated HTS Platform

1. Attractive library

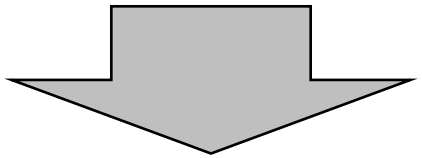
- Pharma origin, huge, high-quality and diverse library

2. State-of-the-art infrastructure

- Fully automated screening systems
- Comprehensive platforms covering diverse target classes and phenotypic screens

3. High quality and comprehensive services

- A proven track record of around 700 HTS campaigns for drug discovery
- Comprehensive services in hit identification including strategy planning, assay development, HTS and profiling
- Hit expansion and lead generation services by highly experienced medicinal chemists
- High-throughput-ADMET profiling services with extensive experience and sophisticated protocols



➢ ***We efficiently offer high-quality hit and lead compounds through our integrated HTS platform***

Together
We can Create a Hopeful Future through Drug Discovery

Axcelead Drug Discovery Partners

Contact@axcelead.com