Biophysical Method and Platform Development for Rapid High Throughput Screening and Hit Validation

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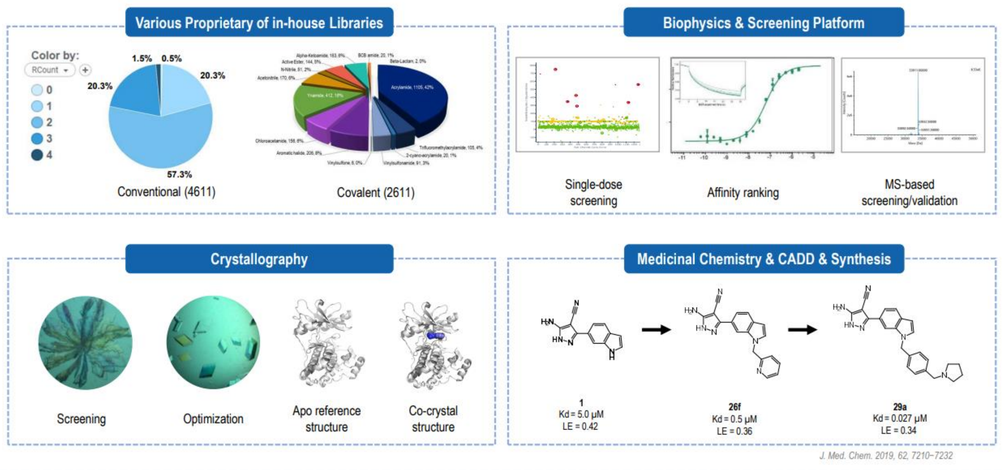
WuXi Biology

Integrated Screening and Validation Framework

An integrated discovery platform combining protein production, proprietary compound libraries, advanced biophysical screening technologies, structural biology, medicinal chemistry, and computer-aided drug design (CADD) enables efficient identification and validation of small-molecule hits. An example case study of a compound library designed for solution-based spectral shift screening was evaluated to identify hits followed by orthogonal validation with surface plasmon resonance (SPR). This workflow supports early-stage hit discovery and downstream hit-to-lead development.



Library Information and Scope

- Rule of 3 Compliant: MW ≤ 300 Daltons, cLogP ≤ 3, HDB and HBA ≤ 3
- Other rules: NRB: ≤3, PSA ≤ 90, stereo ≤ 2, charged groups ≤ 1
- Filters: PAINS, Lilly-filters, REOS, Kazius
- Library is continuously monitored to ensure stability, >90% purity in LC/MS + NMR, solubility, diversity, and continuous synthesis of novel fragments to add to library
- Diverse libraries for both covalent and reversible fragments
- New “Extended Fragment Library” includes >50K fragments and has been optimized for diversity with Tanimoto similarity threshold = 0.75.
- Established precedence for successful screening against RNA, Helicases, Kinases, E3 Ligases, and Transcription Factors

Focused Libraries:

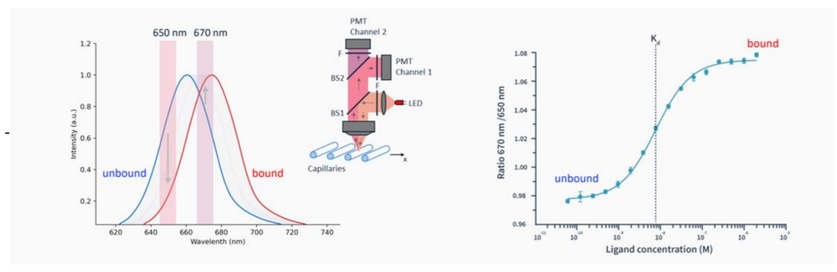
RNA Library	Bioactives Library	Approved Drugs	Natural Products	CNS Library	PPI Library	PPI Fragment
~5,500	~1,300	~2,800	~2,500	~73,000	~48,000	~5,300
GPCR Library	**Protease Library**	**Molecular Glue Degrader Library**	**Kinase Library**	**Covalent Library**	**Covalent_Lysine**	**Covalent_Serine**
~10,000	~13,000	~4,700	~16,000	~69,000	~13,000	~8,000

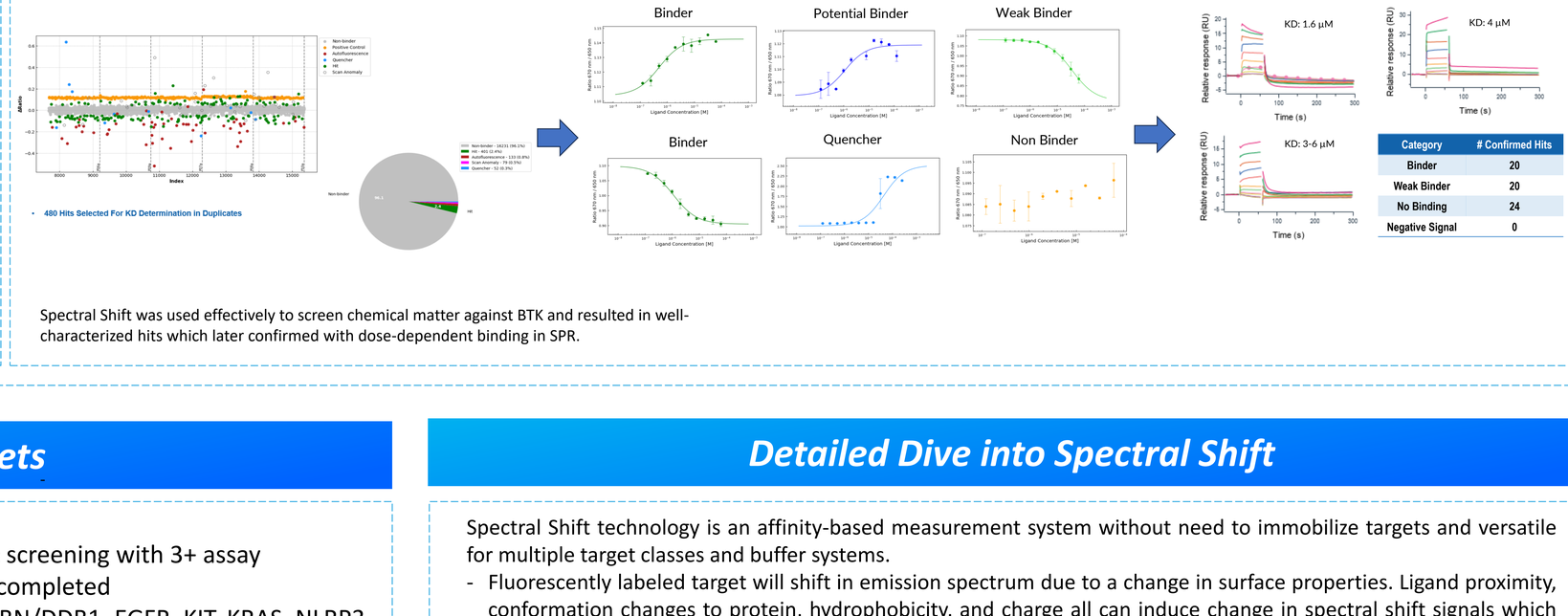
Our HTS Platform Capabilities and Technology

	DNA Encoded Library Screening (DEL)	Fragment Based Drug Discovery (FBDD)	High Throughput Screening (HTS)	Affinity Screening Mass Spectrometry (AS/MS)	Spectral Shift
Screen Capability Scope	1 million – 100 billion	1-10K, 50K extended available	10K- 1 million	10K-1 million	50K (280-350 Da MW)
Time to Complete Screening	4 weeks	3-4 weeks	1-12 months * (scale/methods)	1-3 months * (scale dependent)	1-2 weeks * (optimization and scale dependent)
Data Readout	Affinity DEL/OBOC (one bead, one compound), Functional OBOC	Affinity and Direct Binding	Functional and Affinity	Affinity	Affinity
Advantages	- Low protein consumption - Challenging Target Amenable - Coverage of Novel chemical space - Novel target with no established screening assay, novel binding pocket and novel MoA	- Low molecular weight - Can identify weak binders - Novel target with no screening assay, novel MoA	- Functional readout - Cell-based screening possible - Individual non-pooled compound samples - Miniaturized assay format possible	- Pooled screens (400-1200 compounds/pool) - Label-free - Amenable to any soluble target– protein/RNA/DNA, seeking novel pocket or MoA	- High throughput plate-based - Solution based - Ternary complex amenable
Considerations	- Off-DNA compound synthesis time requirements	- Low initial affinity - Longer lead compound optimization time	- Potential lengthy assay development timeline - Higher hit-finding costs	- Low sensitivity for weak binders	- Dye-based method

Technology Spotlight: Ultra High Throughput Screening (uHTS) with Spectral Shift using Dianthus

Spectral Shift technology is an affinity-based measurement system to characterize binding events between proteins and small molecules. Ligand proximity, conformation changes to protein, hydrophobicity, and change all induce shift in emission spectra of attached fluorescent probe, thereby allowing for detection of small molecule binding to protein.





Category	# Confirmed Hits
Binder	20
Weak Binder	20
No Binding	24
Negative Signal	0

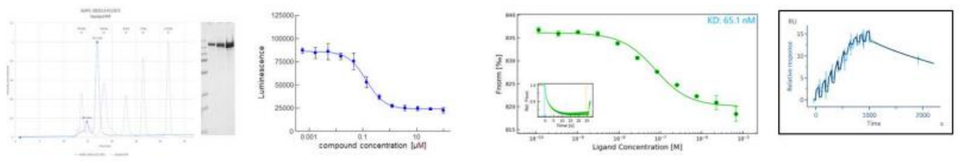
Spectral Shift was used effectively to screen chemical matter against BTK and resulted in well-characterized hits which later confirmed with dose-dependent binding in SPR.

Ready To Go Screening Targets

- Ready to Go Assay Suite– > 60 protein targets are ready for immediate screening with 3+ assay technologies enabled and developed, and protein production already completed
- → Targets include BTK, BLM, BRD2-4, CDK + CDK/CCND complexes, CRBN/DDB1, EGFR, KIT, KRAS, NLRP3, p38, PARP1, PIK3CA/PIK3R1, RECQL, SHP2, STAT 1-6, and WRN

Show case:

Activity-Validated Assays of NLRP3 vs. MCC-950



High quality assay grade protein produced in house
Protein QC: SDS-PAGE, aSEC, MS

ADP-Glo assay
Impact of MCC-950 on NLRP3 activity
IC_{50}: 134 nM

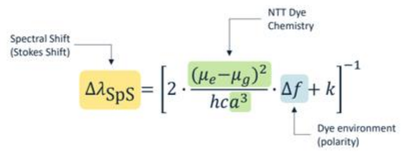
Labeled MST
Binding affinity of MCC-950 to NLRP3
K_D: 65.1 nM

SPR
Binding affinity of MCC-950 to NLRP3
K_D: 20 nM

Detailed Dive into Spectral Shift

Spectral Shift technology is an affinity-based measurement system without need to immobilize targets and versatile for multiple target classes and buffer systems.

- Fluorescently labeled target will shift in emission spectrum due to a change in surface properties. Ligand proximity, conformation changes to protein, hydrophobicity, and charge all can induce change in spectral shift signals which thereby allows for detection of small molecule binding.
- Spectral shift signal is recorded as the ratio between 2 wavelengths



- Can measure the radiometric signal of a labelled target as a function of ligand concentration to obtain a dose response curve.
- Fast reads– amenable to 1536 well plates --> 370K compounds can be screened in 2 weeks
- uHTS SpectralShift platform offers an alternative for classical HTS for enzymatically inactive proteins
- Can measure ternary complex formation and can enable HTS screening for the identification of bifunctional molecules/ molecular glues
- uHTS Dianthus can be used to develop a Ki/Kinact for up to 64 compounds in 1536 well plate. Previous data has shown good alignment with MS measurements.

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