Unleashing the Power of Spectral Shift Technology for Ultra-High-Throughput Binding Assays

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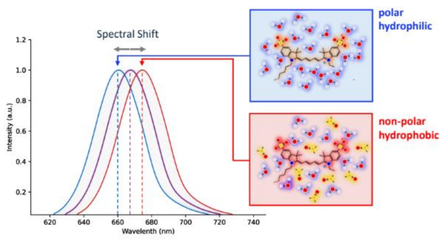
Abstract

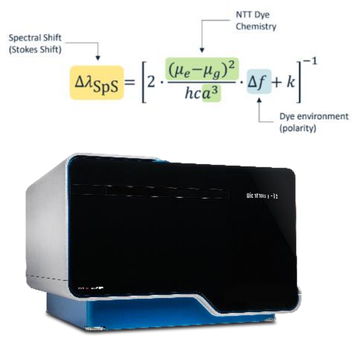
High-throughput screening (HTS) remains critical in early drug discovery, yet traditional biochemical assays and ASMS can be limited by sensitivity or sample demand. We present a Spectral Shift–based direct binding assay implemented using NanoTemper Technologies' Spectral Shift technology, enabling detection of protein–ligand interactions with high sensitivity and low material consumption.

We describe an optimized, high-throughput workflow and demonstrate robust performance across kinase targets including BTK, CDK2, and PIK3CA H1047R. These results highlight Spectral Shift as a versatile HTS approach for accelerating early discovery decisions.

Spectral Shift Technology

Spectral Shift–based direct binding assays for ultra high-throughput screening





Technology Background:

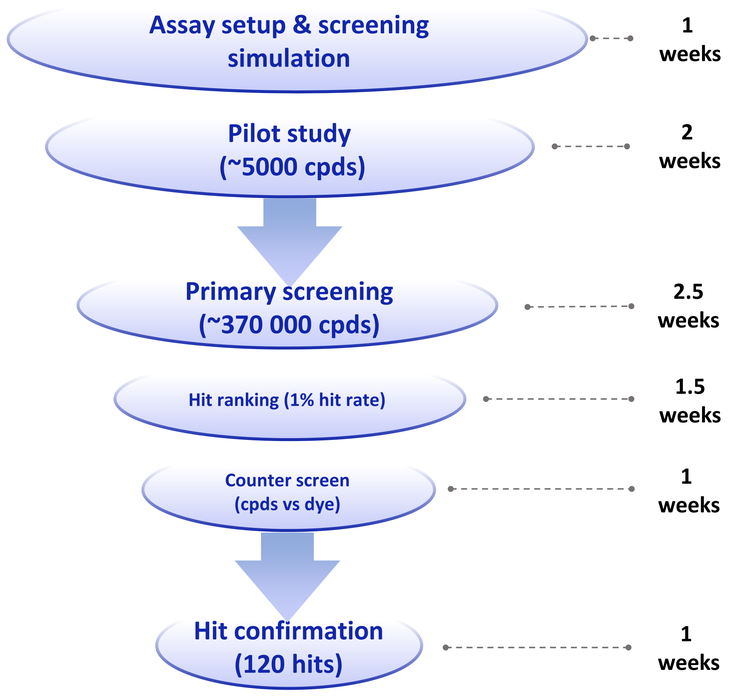
- The polarity of the environment that surrounds a fluorophore affects its emission spectrum
- Binding events change the surface properties & induce a shift in the emission of the attached fluorescent probe
- Ligand proximity, conformation changes in protein, hydrophobicity & charge will affect the Spectral Shift signal & will allow to detect binding of a small molecule to a protein

Dianthus™ uHTS with Spectral Shift Technology:

- **Affinity Measurements** in free solution at isothermal conditions
- **High Sensitivity** enables studying all target and ligand classes
- **Very Fast**: one **1536 well plate in 5 min** - 370k small molecules in 2 weeks

Workflow

Workflow at Crelux, part of WuXi AppTec, to support Dianthus uHTS screenings

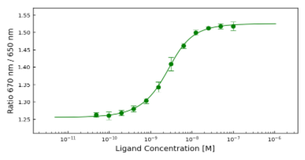


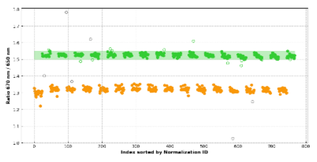
The fully automated screening workflow supports diverse target classes and molecular modalities, enabling **screening of libraries of up to 1 million compounds within approximately 2 weeks**. A complete end-to-end workflow—including assay development, validation, and execution—is established within ~9 weeks for any screening campaign. The workflow is designed to accommodate multiple molecule types and target families, delivering robust, scalable performance with rapid turnaround for high-throughput discovery programs.

Results

Results generated by Crelux, part of WuXi AppTec

1. PIK3CA H1047R : Assay Setup & Screening Simulation



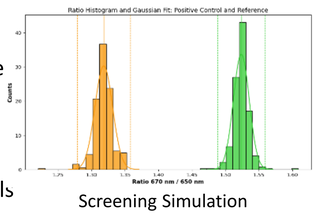


Assay Optimization:

- Tested labelling strategies
  NHS, Maleimide, RED-tris-NTA
- Various assay buffers & additive
  pH, salt, reducing agents

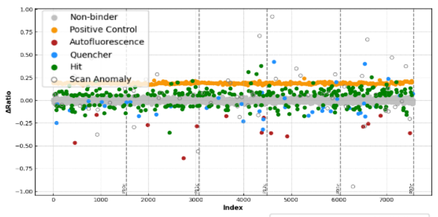
Assay robustness:

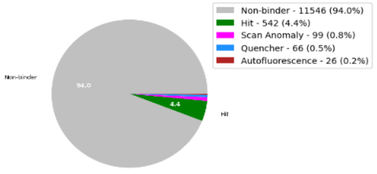
screening simulation on 1536 wells



Screening Simulation

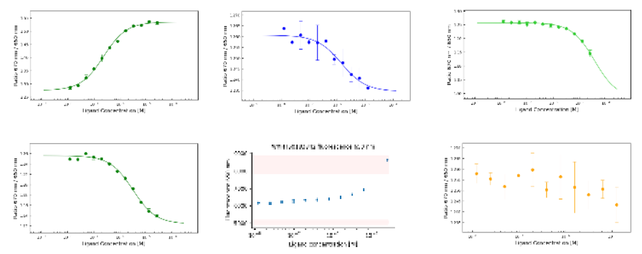
2. PIK3CA H1047R: Primary Screening





639 Hits Selected for K_D Determination in 12-point Duplicates

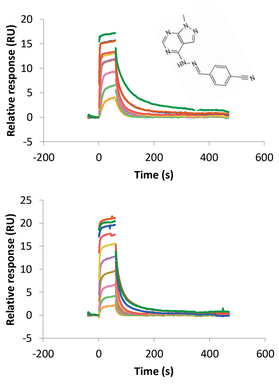
3. PIK3CA H1047R: Hit Confirmation & Characterization



Category	
Binder	217
Potential Binder	13
Weak Binder	211
Quencher/Autofluor.	2
Non Binder	197

64 Best binders selected for orthogonal validation by SPR

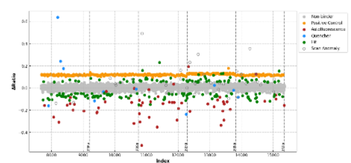
4. PIK3CA H1047R: Orthogonal Confirmation by SPR

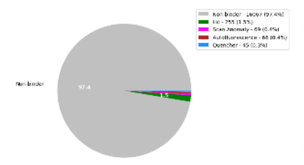


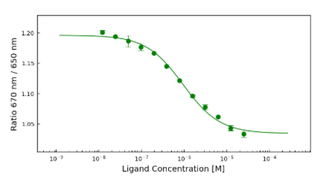
Category	
Binder	38/64
Weak Binder	15/64
No Binding	11/64
Negative Signal	0/64

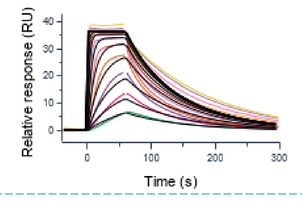
Summary: HTS Screening of BTK, PIK3CA, CDK2

	CDK2	BTK	PIK3CA H1047R
Hit Rate	2.4%	2.4%	4.4%
KD Range SpS	0.7 -50 µM	0.2-50 µM	0.05-50 µM
Orthogonal Validation (%)	39%	62%	82%
Robust Z-Score	0.81	0.65	0.75









Bibliography

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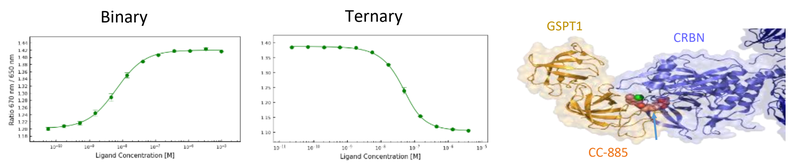
Summary

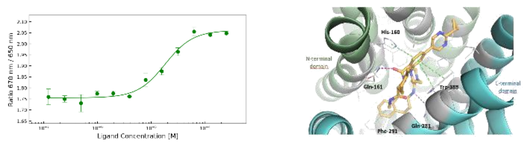
The Spectral Shift Dianthus uHTS platform by NanoTemper is fully integrated into the Biophysics Hit Profiling and Identification workflow at WuXi Biology, enabling direct, high-throughput binding measurements within a standardized screening framework. Spectral Shift technology provides a powerful alternative to traditional biochemical assays and ASMS by directly quantifying protein–ligand interactions in a high-throughput format.

Dianthus uHTS by NanoTemper supports a broad range of target classes, including membrane proteins, transcription factors, and protein–protein interactions, and is compatible with diverse and emerging drug modalities such as PROTACs, molecular glues, fragments, covalent inhibitors, and peptides. This flexibility enables robust support for a wide range of hit-to-lead discovery programs.

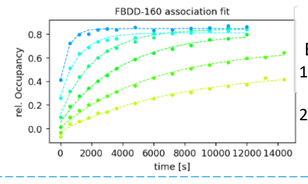
Target Protein Degradation



Membrane Proteins (SLC15A4)

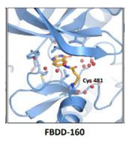


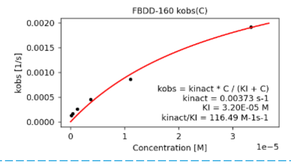
Covalent Molecules: kinact/KI for BTK



BTK vs. Covalent Fragment

1. Obtain observed association rate k_{obs}
2. Determine k_{inact}/K_I from $k_{obs}(C)$





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