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

WuXi Biology

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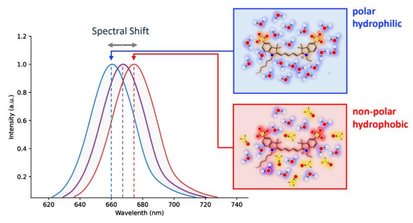
Abstract

High-throughput screening (HTS) is vital in drug discovery, yet traditional methods like biochemical assays and affinity selection mass spectrometry (ASMS) face potential limitations. We introduced a spectral shift-based direct binding assay that enhances precision, sensitivity, and efficiency while reducing sample consumption and turnaround time. In this poster, we present the underlying technology, detail our in-house implementations and optimized screening workflows, and highlight a successful case study targeting PIK3CA H1047R. The results demonstrate the assay's robust performance and broad applicability in accelerating early-stage drug discovery.

Spectral Shift Technology:



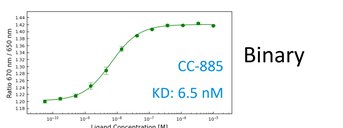
Technology Background:

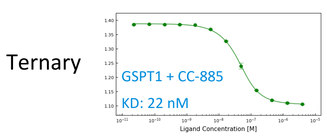
Ligand binding to a labeled target changes the surrounding properties & induces a **shift in the emission of the fluorescent probe**

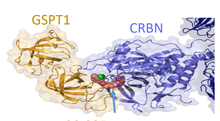
uHTS Dianthus - Spectral Shift Technology:

- **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

Targeted Protein Degradation

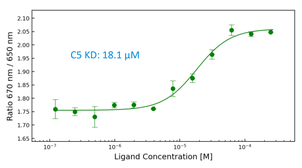


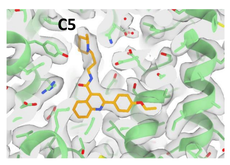




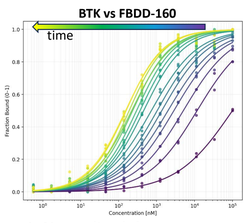
Membrane Proteins

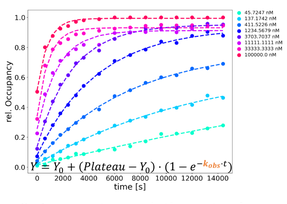
SLC15A4

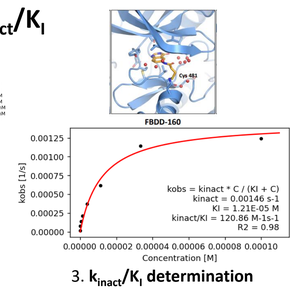




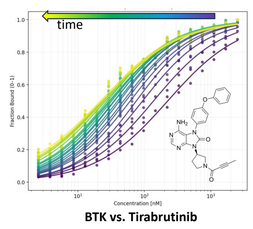
Covalent Molecules: k_{inact}/K_I



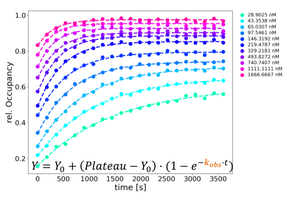


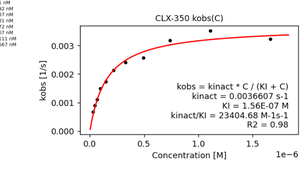


1. Normalized **dose resposes** → 2. Apparent **association rates k_{obs}** → 3. **k_{inact}/K_I determination**



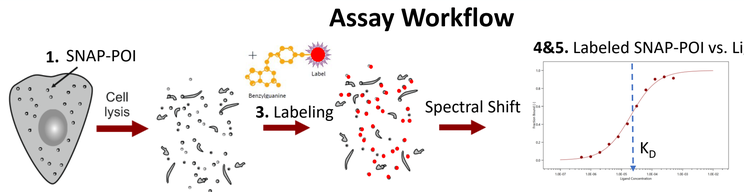
BTK vs. Tirabrutinib

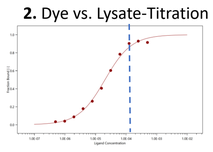




Cellular Biophysics: Purification-Free Spectral Shift Assays in 293S Cell-Lysate

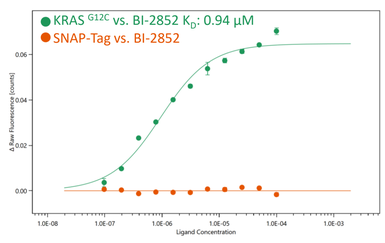
Assay Workflow



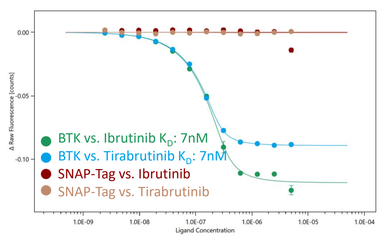


1. **Expression of SNAP-tag-POI** and SNAP-tag control in 293S cells & cell lysis
2. **Assess lysate concentration** for 1:1 in-lysate labeling: dye vs. lysate-titration
3. **Labeling of SNAP-POI** & SNAP-tag control with SNAP-specific benzylguanine dye
4. **Incubation of ligand dilution series** & labeled lysates
5. **Spectral Shift data acquisition → K_D**

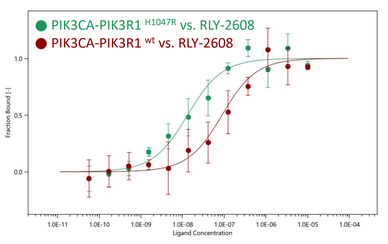
SNAP-KRAS ^{G12C} vs. BI-2852



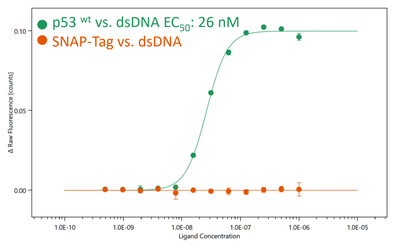
SNAP-BTK vs. covalent small molecules



SNAP-PIK3CA ^{H1047R} vs. RLY-2608



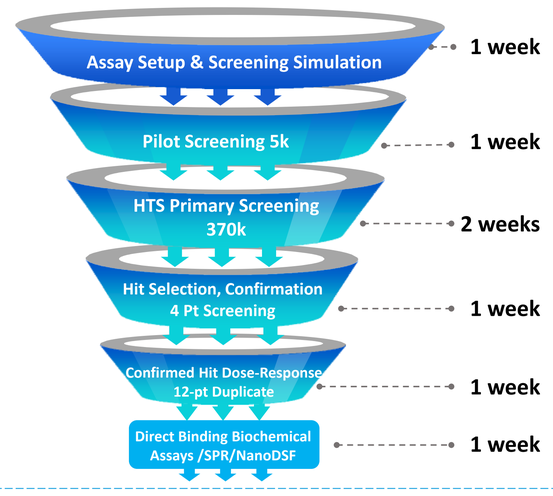
SNAP-p53 ^{wt} vs. dsDNA



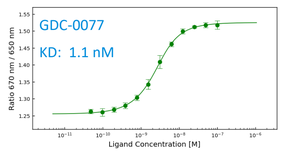
- **Biophysical Binding Assays in Near-Native Conditions: purification free sample preparation meets isothermal data aquision in solution**
- **Potential Applications: HTS screenings, efficiency of covalent binders: k_{inact}/K_I, mode-of-action studies, functional assays, membrane proteins**

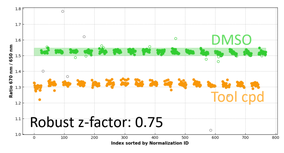
Dianthus uHTS – Screening Workflow Showcase Using PIK3CA ^{H1047R} vs. WuXi AppTec Kinase Focused Library (16.5 k)

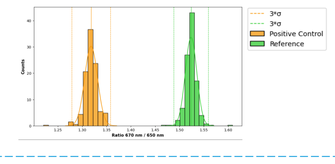
Dianthus uHTS Workflow



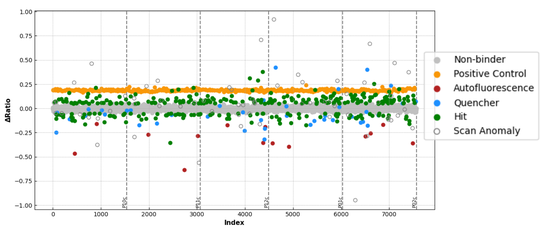
Assay Setup & Screening Simulation

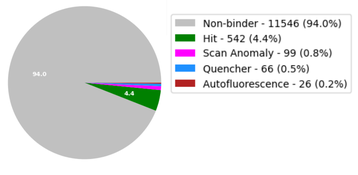






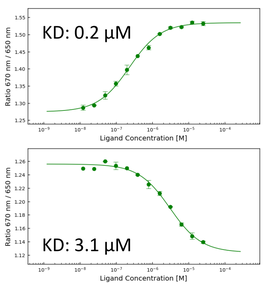
Single-Point Primary Screening





639 Hits Selected for K_D Determination

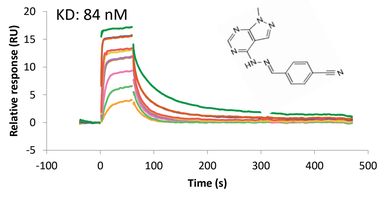
12-Point Hit Confirmation



Category	#
Binder	217
Potential Binder	13
Weak Binder	211
Quencher/ Autofluorescence	2
Non Binder	197

64 Hits Selected for Orthogonal Validation

Orthogonal Confirmation by SPR



82% of Best SpS Hits Confirmed

Outlook

- **Automated** sample preparation increases throughput
- **HTS screening platform** for any sample type
- **Mode-of-action studies** in lysate vs purified protein
- **k_{inact}/K_I** determination In lysate vs purified protein

Summary

- Spectral Shift uHTS Dianthus platform is fully integrated into the Biophysics Hit ID and Characterization Workflow at WuXi Biology. This versatile system supports a broad range of targets and assay conditions, offering a powerful alternative to classical HTS—especially for challenging proteins, including detergent-solubilized membrane proteins. It excels in detecting ternary complex formation and enables screening for molecular glues and bifunctional molecules.
- In-lysate screening using uHTS Dianthus offers near-native conditions for various applications such as HTS screening, covalent ligand profiling, and mode-of-action studies.
- To exploit the method's full potential, robust assay design—particularly protein labeling—is critical and must be tailored to each target.
- In future, machine learning tools will accelerate analysis of the large datasets generated and improve both speed and user experience.

References

Spectral Shift: A New Spectral Shift-Based Method to Characterize Molecular Interactions: Andreas Langer, Annemarie Lüdecke, Tanja Bartoschik, Ondrej Cehlar, Stefan Duhr, Philipp Baaske, and Werner Streicher; ASSAY and Drug Development Technologies 2022 20:2, 83-94; DOI: 10.1089/adt.2021.133

Covalent compound analysis (kinact/KI): Strelow JM. A Perspective on the Kinetics of Covalent and Irreversible Inhibition.; SLAS DISCOVERY: Advancing the Science of Drug Discovery. 2016;22(1):3-20. doi:10.1177/1087057116671509

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