The Application of Spectral Shift to Drug Discovery Projects

Domainex
Enrich your Medicines Pipeline

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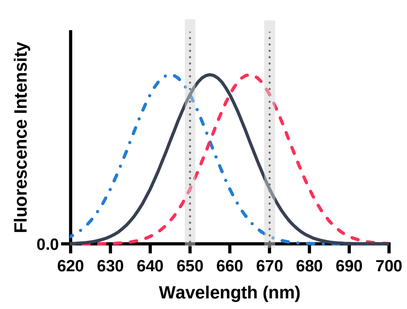
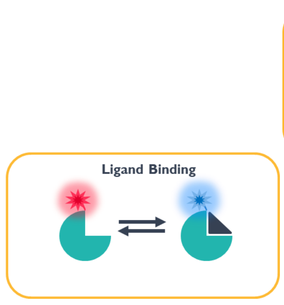
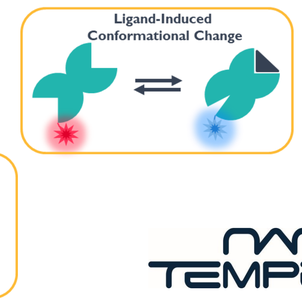
Introduction

- Spectral Shift is a fluorescence-based biophysical technique used to determine ligand binding
- The assay requires modification of a target protein with NanoTemper's 2nd generation RED dye
- The dye's chemical environment can be affected directly when the ligand binds in close proximity or through confirmational changes induced by ligand binding, which cause a shift in the emission wavelength
- K_Ds are derived by plotting the ratiometric measurement (FI 670 nm / 650 nm) against ligand concentration
- Spectral Shift generally boasts a better signal:noise ratio than MST and TRIC with less sensitivity to aggregates
- This poster highlights Domainex's expertise in Spectral Shift and our ability to produce innovative high-quality assays







NanoTemper

Fragment Screening

Polymer-Encapsulated Nanodiscs

- G protein-coupled receptors (GPCRs) are important drug discovery targets and are a challenging class of proteins to produce and purify in multi-milligram quantities
- Domainex offer an alternative, detergent free strategy to purify membrane proteins using synthetic polymers that solubilize cell membranes and spontaneously form nanodiscs containing native lipids and proteins

Assay Development:

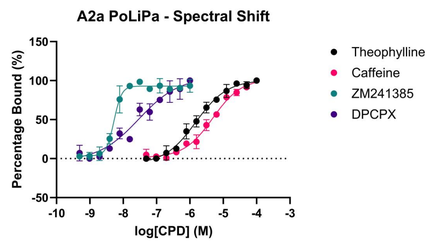
- The A2a receptor, a class A GPCR, was purified using a polymer-encapsulated nanodisc
- A2a was fluorescently labelled using a common strategy from NanoTemper
- Several known small molecule antagonists were then used to validate the Spectral Shift assay

Fragment Screening:

- Following assay validation, a fragment screen was performed using Domainex's fragment library
- Theophylline was used a positive control during the screen
- A total of 125 initial hits were identified from the single dose screen

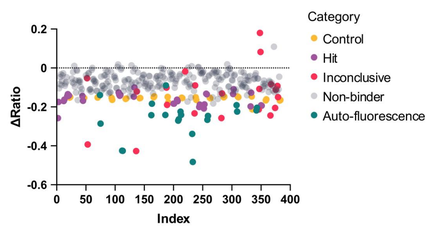
Hit Validation:

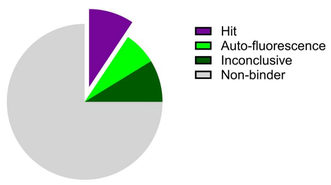
- A second single dose screen was performed at a lower concentration to eliminate weak binders
- Affinity determination was performed for the best binders
- 19 promising hits were identified with ligand efficiencies over 0.4

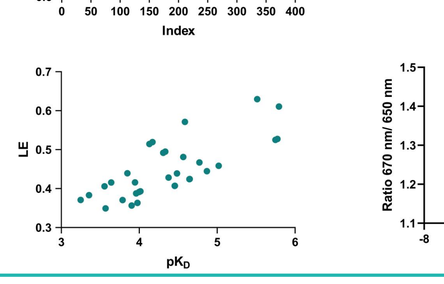


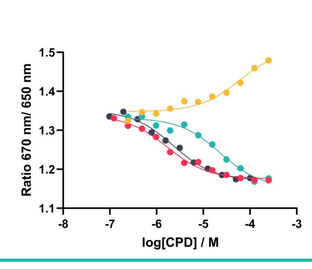
Compound	Hill Slope	Spectral Shift pK_D	Literature values^ (pK_i)
Caffeine	1.01	5.3	4.6-5.6
Theophylline	0.92	5.8	5.2-5.8
DPCPX	0.72	7.5	6.6-7.2
ZM241283	3.75*	8.3	8.8-9.1

*at the assay tight binding limit
^IUPHAR/BPS Guide to pharmacology website https://www.guidetopharmacology.org/









	pK_D	LE
DMX0009121_2	4.13	0.52
DMX0009151_2	4.59	0.58
DMX0009217_2	5.75	0.52
Theophylline	5.80	0.62

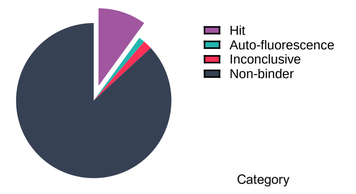
$LE = 1.37*(pK_D)/HAC$

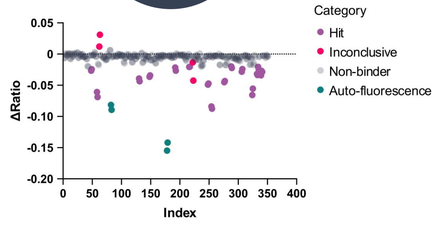
RNA

- RNA adopts a variety of highly ordered 3D structures which perform different biological functions
- Targeting RNA could provide an alternative therapeutic strategy for treating 'undruggable' proteins through preventing their synthesis
- Non-coding RNA could also be implicated in certain diseases through regulation of cellular signaling pathways or translation of coding RNA
- Domainex have successfully performed a fragment screen against an RNA target using Spectral Shift

Fragment Screening:

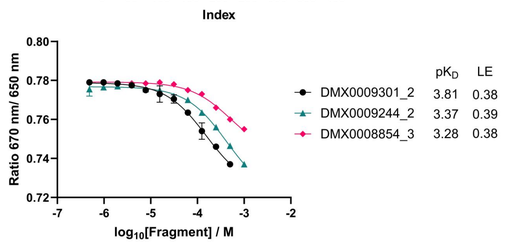
- Fluorescently labelled RNA was subject to a folding protocol
- As no positive controls were available for assay validation the fragment screen was initiated
- A total of 112 hits were taken forward to affinity determination





Hit Validation:

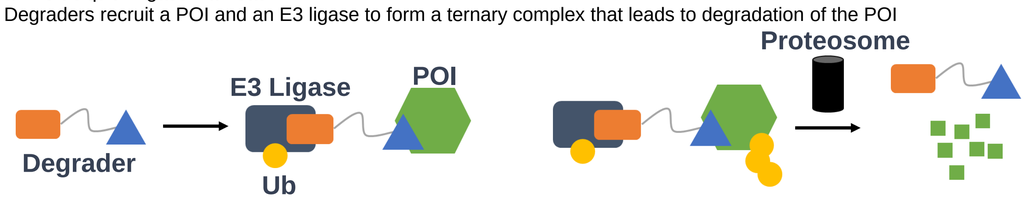
- Affinity determination for the fragments hits was performed
- Three fragments were identified with affinities less than 600 µM and promising ligand efficiencies



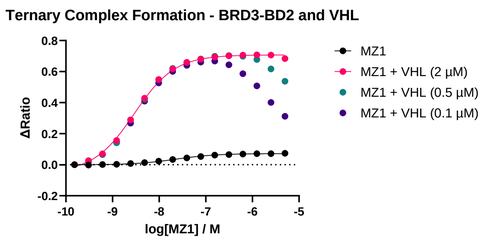
	pK_D	LE
DMX0009301_2	3.81	0.38
DMX0009244_2	3.37	0.39
DMX0008854_3	3.28	0.38

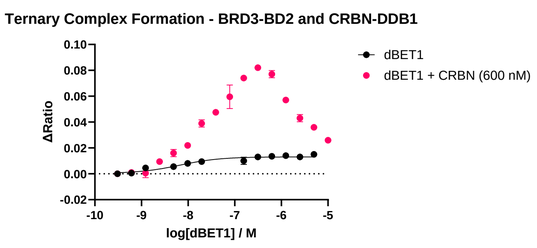
Ternary Complex Formation

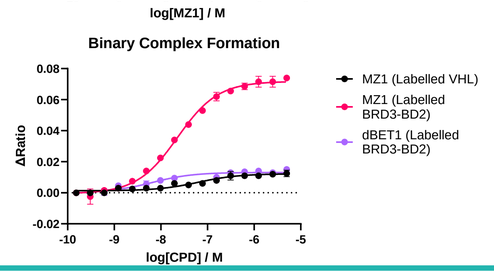
- Degrader drugs are heterobifunctional molecules with three components: a protein-of-interest (POI) binding moiety, a linker and an E3 ubiquitin ligase warhead
- Degraders recruit a POI and an E3 ligase to form a ternary complex that leads to degradation of the POI



- Spectral Shift can be used to generate binary affinities, ternary affinities and alpha values (cooperativity)
- MZ1 and dBET1; degraders that connect a ligand for BRD3-BD2 (or other BRD proteins) and either VHL or CRBN warheads, were used to showcase Spectral Shift
- BRD3 is a member of the Bromodomain and Extra-Terminal motif (BET) protein family and a therapeutic target for various diseases including cancer
- Cereblon (CRBN) and von Hippel-Lindau (VHL) are commonly utilized E3 ligases in degrader development
- BRD3-BD2, VHL and CRBN proteins have all been prepared by Domainex for use in this study







Degrader	Binary K_d / nM	Ternary K_d / nM	α value
MZ1	23	3	7.6
dBET1	7	24	0.3

Kinase Competition Assay

Challenge:

- Fluorescently labelling proteins are strategy and construct dependent
- Labelling can have a destabilizing effect, cause conformational changes or block binding sites
- The site of the label can limit assay sensitivity

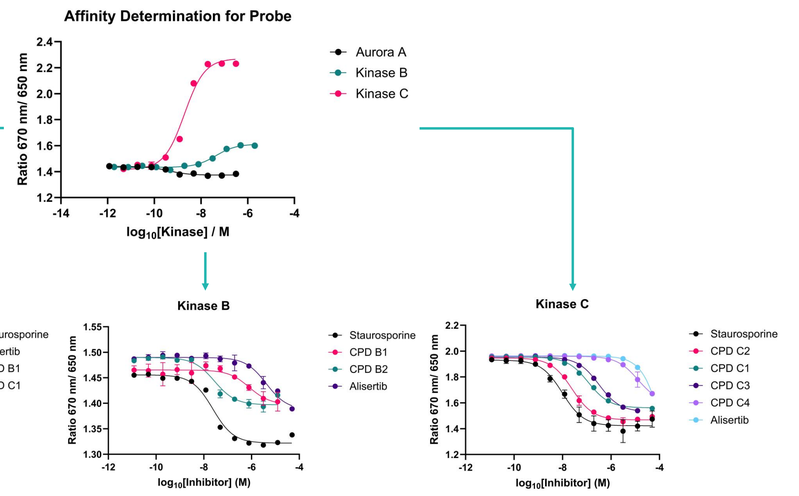
Solution:

- If direct binding assay is not compatible with the POI, a competition assay could be developed
- Spectral Shift provides an alternative to AlphaLISA, HTRF and FP

Conclusion:

- A competition assay was successfully developed for ATP-competitive inhibitors for several kinases
- IC50 values within 2-fold of FP values and assay does not have the same drawbacks as FP

CPD	Aurora IC50 / nM		Kinase B IC50 / nM		Kinase C IC50 / nM	
	SS	FP	SS	FP	SS	FP
Staurosporine	7	7	24	16	10	13
CPD B1	NB	NB	802	1331	-	-
CPD B2	-	-	28	16	-	-
Alisertib	1	1	4141	3109	>50000	27480
CPD C1	NB	NB	-	-	117	148
CPD C2	-	-	-	-	25	35
CPD C3	-	-	-	-	317	505
CPD C4	-	-	-	-	13030	26570



Summary

- ✓ We believe Domainex has performed the first fragment screen with a membrane protein purified in a Polymer-Encapsulated Nanodisc
- ✓ RNA binders have been identified from our fragment library using Spectral Shift
- ✓ We have developed TCF assays for the two most common E3 ligases in degrader research
- ✓ A fluorescently labelled competitive inhibitor was used to develop a competition assay providing an alternative when protein labelling is not possible
- ✓ Domainex can utilize Spectral Shift to support a wide range of drug discovery projects and modalities

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about our drug-discovery platform, please contact: **enquiries@domainex.co.uk**

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