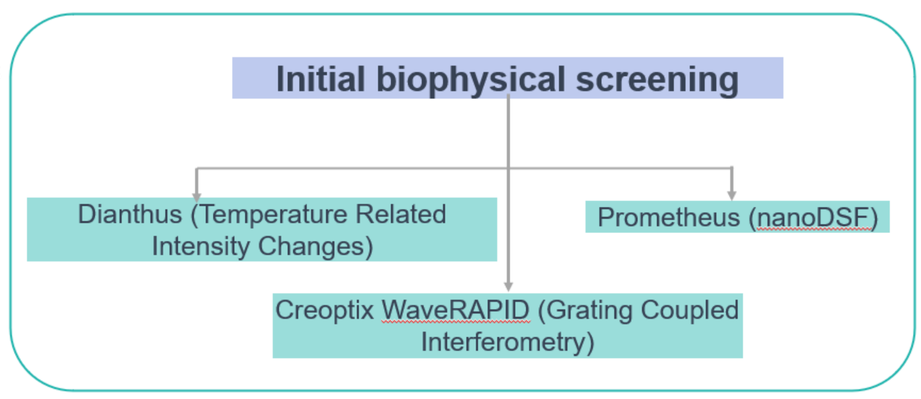
New kids on the block: Fragment screening using Temperature Related Intensity Change and Grating Coupled Interferometry

Domainex
Enrich your Medicines Pipeline

Kartika Shetty, Alec O`Keeffe, Christopher Morton, Trevor Askwith and Jana Wolf
Domainex Ltd, Chesterford Research Park, Little Chesterford, Saffron Walden, Essex, CB10 1XL

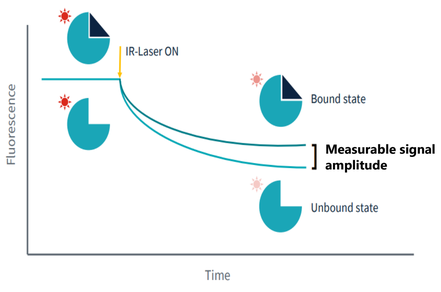
Biophysical assays have revolutionised the drug discovery process and utilisation of biophysical methods has become increasingly high for hit-finding of target classes previously considered as “undruggable”. Fragment screening for hit identification has become increasingly popular due to significant advances in technology. Domainex has invested in two disruptive technologies that drastically increase the throughput of quantitative, biophysical assays**: Grating Coupled Interferometry (Creoptix Wave)** and the **Dianthus (NanoTemper Technologies)**.

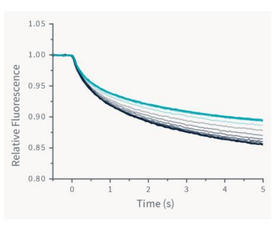
Here we describe initial primary fragment screening for a large protein (120 kDa) with high resolution, employing both techniques (along with nanoscale Differential Scanning Fluorimetry (nanoDSF)) through hit validation and further into hit expansion activities



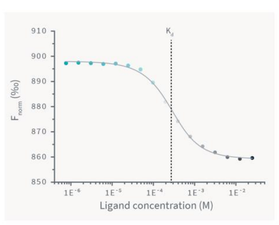
Technical background: Dianthus

Plate based in-solution binding assay, which employs Temperature Related Intensity Changes (TRIC) in fluorescence intensity to quantify ligand binding. The Dianthus is suitable for single concentration screening and the determination of true K_D values. The sensitivity of the Dianthus pico detector allows for assaying at low nanomolar to high picomolar concentrations of protein. This means the Dianthus assays can scale in a way many other biophysical approaches cannot.





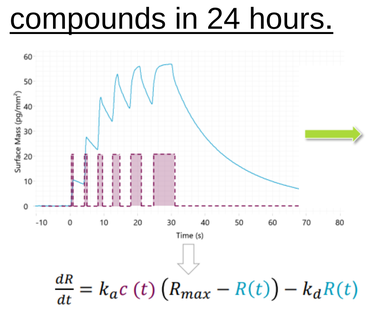
Relative fluorescence measured is plotted against time for each target plus ligand dilutions



Change in fluorescence is measured and plotted against ligand concentration to obtain dissociation constant or K_d

Technical background: Grating Coupled Interferometry (GCI)

Analogous to Surface Plasmon Resonance (SPR) however, the laser light travels over the entire length of the chip rather than a single point and the novel fluidics enables measurements of very fast off-rates up to 10 s^{-1}. This increases the sensitivity as more binding events contribute to the overall signal, which is ideal to identify weakly bound fragment hits. The newly launched waveRAPID technology increases system throughput and compound handling time allowing full kinetic characterisation with unattended loading capability for up to 400 compounds in 24 hours.



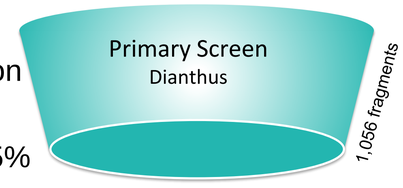
$$\frac{dR}{dt} = k_a c\,(t)\,(R_{max} - R(t)) - k_d R(t)$$

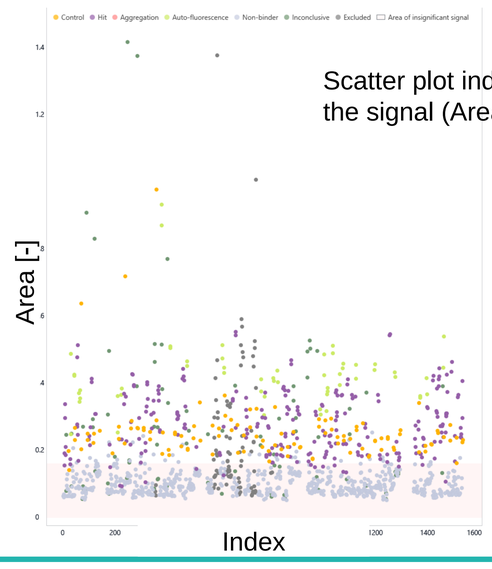
k_{on}
k_{off}
K_D
R_{max}

While traditional methods rely on injections of different analyte concentrations from different wells, waveRAPID uses varying injection durations from a single well to achieve a time dependent concentration function (c(t)), which is then used in an ordinary differential equation to determine kinetic parameters

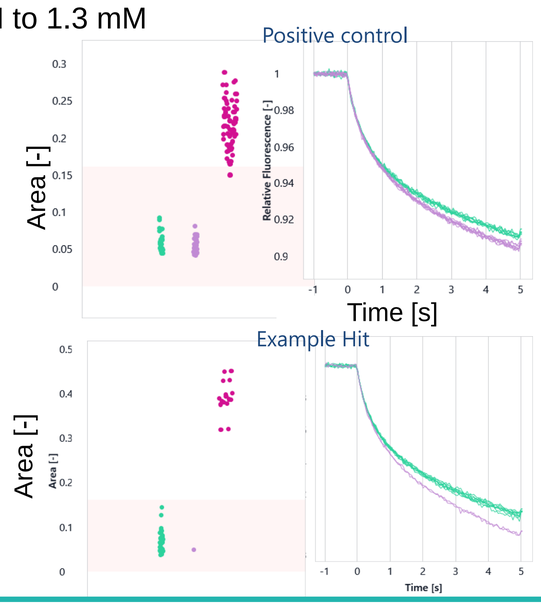
Primary Screen using Dianthus

- Library of 1056 fragments, screened at single concentration of 250 µM in duplicate
- 142 initial hits identified based on a cut-off of ΔFnorm =3.5%
- Concentration response curves confirmed 106 binders (**10% hit rate**) whose affinity ranged between 1.5 µM to 1.3 mM



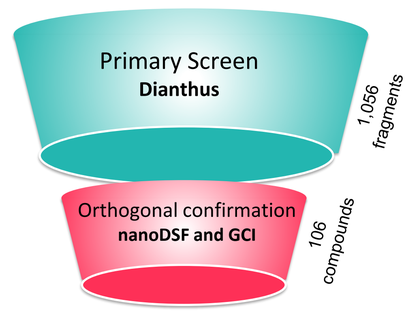


Scatter plot indicating the signal (Area) .



Orthogonal confirmation using NanoDSF and GCI (RAPID mode)

- 106 hits screened using nanoDSF and GCI via (RAPID kinetic mode)

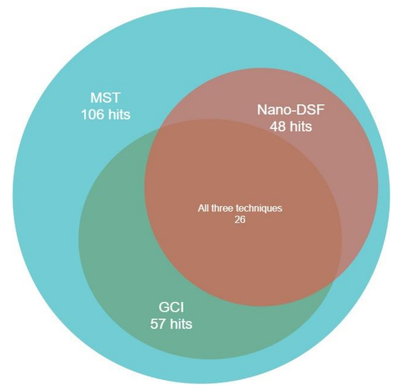


NanoDSF

- Compounds tested at single concentration in duplicate and 49 confirmed as binders (**48.5% confirmation rate**)

GCI

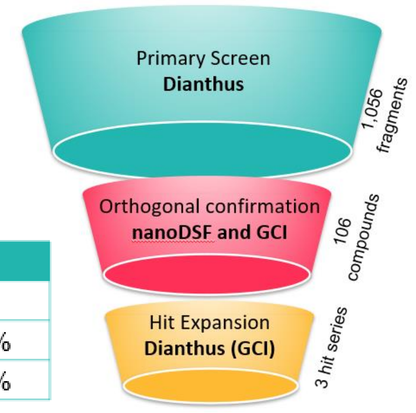
- High immobilisation achieved to measure fragment binders
- 57 compounds confirmed, 54% confirmation rate



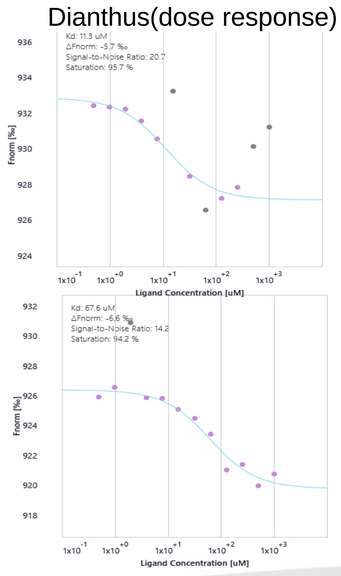
- 79 hits confirmed in 2 techniques with Ligand efficiency (LE) >0.3
- 5 hits LE >0.6 (more promising starting points)
- 3 series identified

Hit Expansion Phase (using Dianthus and GCI)

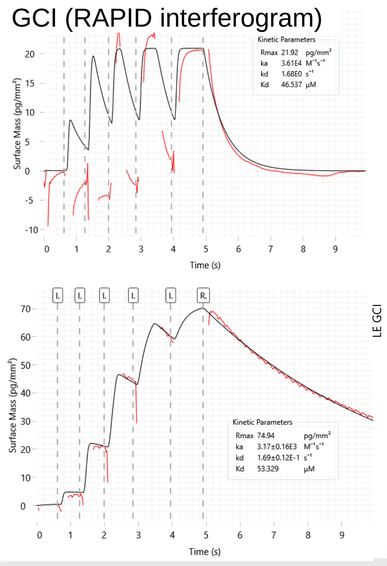
- 105 closely related analogues were purchased across 3 hit series (A, B and C)
- Strong correlation between both techniques builds further confidence in compound series (all 3 series validated)



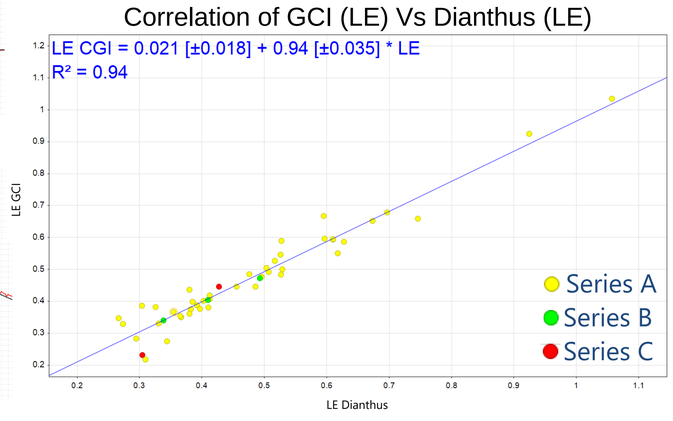
Dianthus(dose response)



GCI (RAPID interferogram)



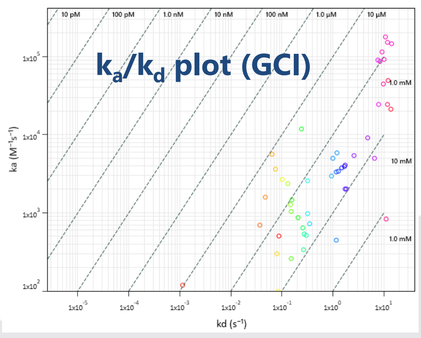
Correlation of GCI (LE) Vs Dianthus (LE)



	Total	%
Analogues tested	105	
Binders	74	70%
Non-binders	31	30%

Summary

Domainex has invested in two cutting edge biophysics technologies (GCI and Dianthus) which have enabled rapid triaging of compounds through primary screen and orthogonal hit confirmation



Services/Contact

If you would like to learn more about applying our drug-discovery platforms, please contact:
enquiries@domainex.co.uk

domainex.co.uk