Successful Fragment library screening by Grating Coupling Interferometry (GCI) against E3 Ligase Target

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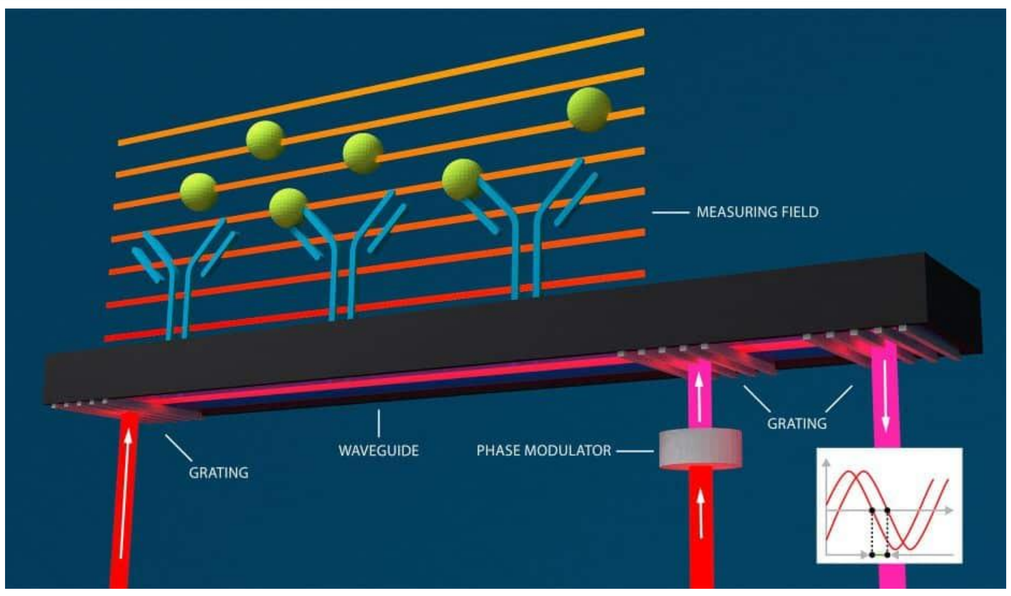
Summary: Domainex has invested in the Creoptix wave Delta instrument. Here we show that the new waveRAPID technology facilitated the screening of Domainex's Fragment Library in significantly shorter screening times when compared to traditional SPR while maintaining excellent data resolution even at large ligand:analyte MW ratios. This technology is exemplified through a FragmentBuilder campaign yielding several series and a crystal structure of ligand bound to the target protein for development.

Grating-coupled interferometry (GCI)

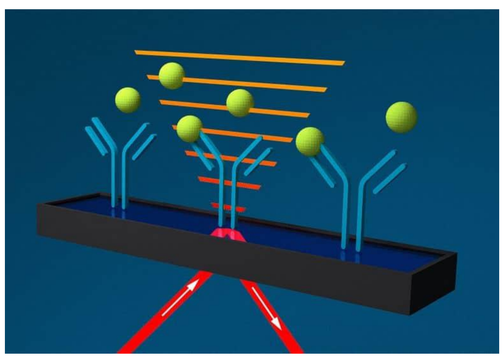
- Binding affinities are measured between a surface bound molecule and substrate in solution, analogous to Surface Plasmon Resonance (SPR).
- Binding of analytes results in mass change in the refractive index within the evanescent field near the chip surface.
- Refractive index changes on a sensor surface are measured as time-dependent phase-shift signals.
- Binding is measured across the whole surface of a chip, meaning better sensitivity vs SPR as the effect of more binding events is calculated.
- Because more binding events are taken into account faster on/off rates can be determined as well as low affinity binding, particularly relevant in fragment based drug discovery.

RAPID (Repeated Analyte Pulses of Increasing Duration)

- In RAPID mode the time of analyte injection is varied rather than the concentration, as seen in traditional binding studies. This allows K_ds to be measured without extensive sample prep and reduces the run time.
- The observed binding curve is a response to the time-dependent concentration input of the injected analyte.
- RAPID allows screening to be run 10x faster than traditional kinetic methods.



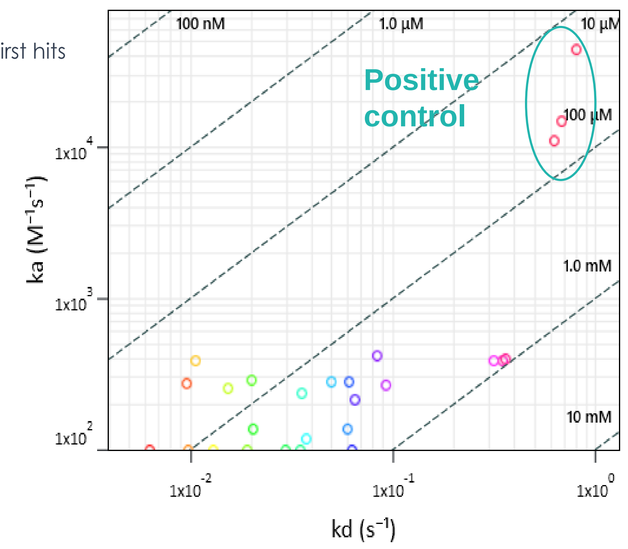
Above: Representation of Grating-coupled interferometry (GCI) showing path of light across entirety of chip taking into account majority of binding events.
Right: Representation of Surface Plasmon Resonance (SPR) showing single point irradiation therefore fewer binding events are measured.
Below: Comparison of typical screening cascades using GCI RAPID and SPR.



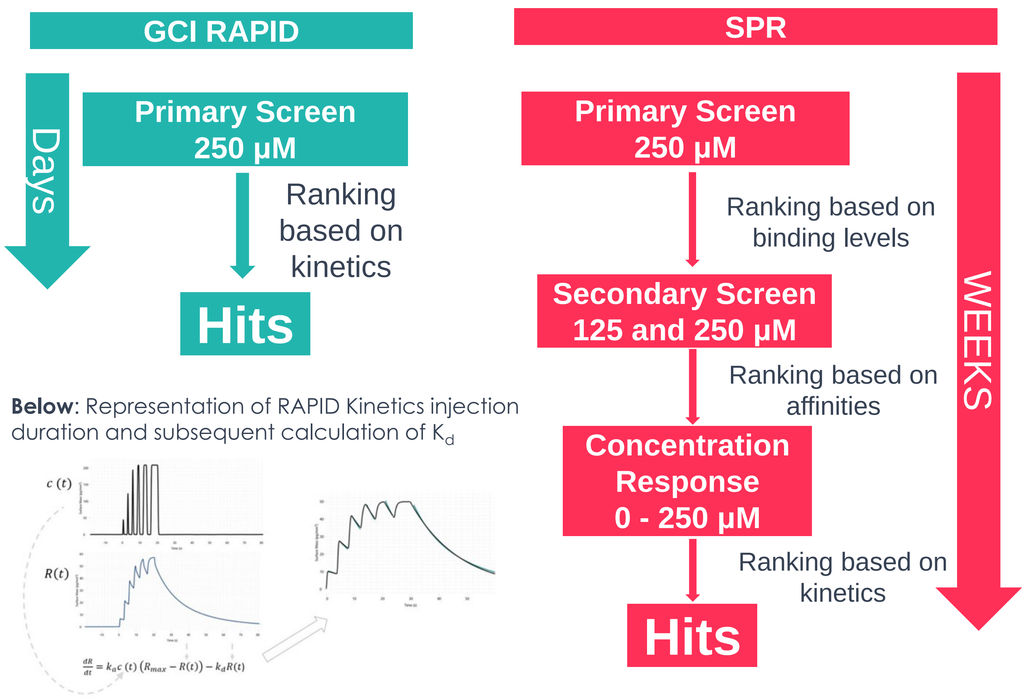
FragmentBuilder

Domainex's fragment library of ~1100 diverse fragments were screened in RAPID mode at 250 µM against the full length (FL) E3 ligase target a truncated construct and a closely related family member. Hits were validated by repeat screening using Traditional Kinetics.

Right: Graphical representation of first hits



However no discernable difference was revealed between the two techniques further confirming the benefit of RAPID. This comparison was made several times over the course of the project. 30 Hits with K_d < 150 µM were taken forward.

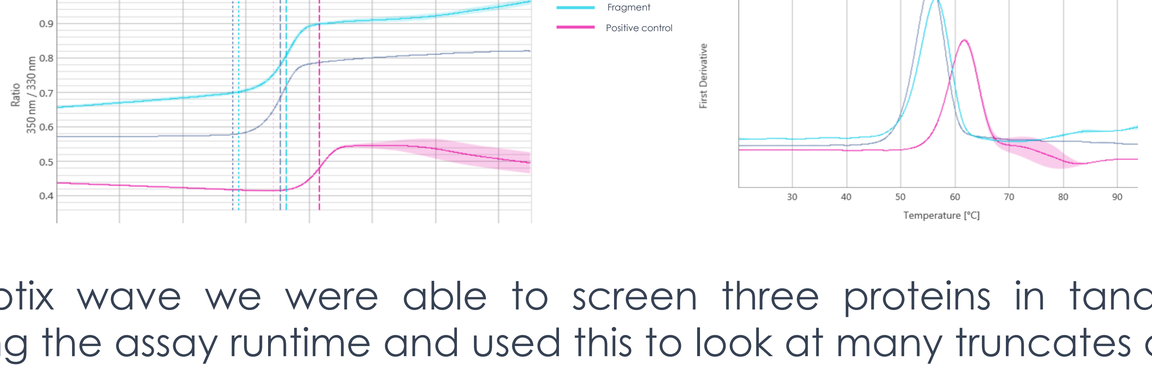


Below: Representation of RAPID Kinetics injection duration and subsequent calculation of K_d

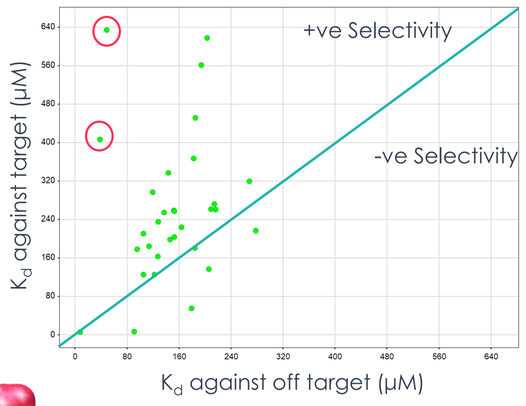
Medicinal Chemistry

Hit expansion was conducted through both synthesis and using commercially available compounds to establish a number of highly related compounds with consistent binding. SAR probing groups were then investigated to indicate where further potency gains could be made. Several series were established with different binding to the FL vs truncates.

Right: Hits were validated by thermal shift using NanoDSF

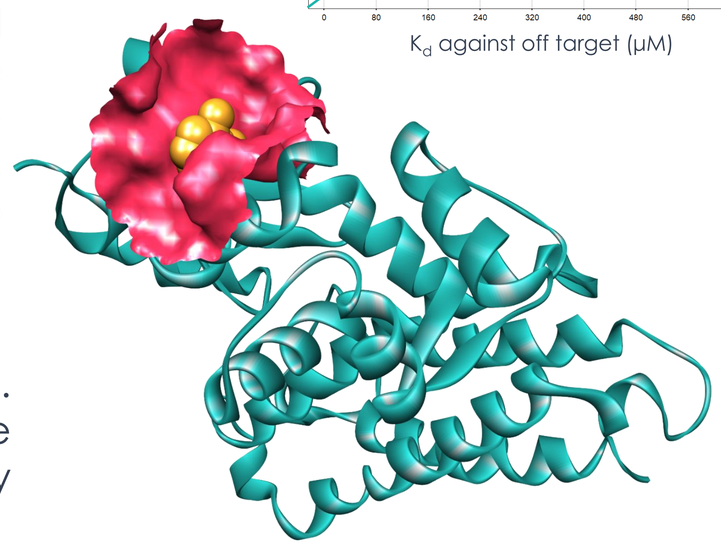


Right: Plot of K_d against target vs K_d of off target. As three proteins can be run in tandem, selectivity and binding information could be collected in one assay expediting development of key fragments.
Below: Crystal structure of E3 ligase with fragment bound.



Assay Biology

Using the Creoptix wave we were able to screen three proteins in tandem without extending the assay runtime and used this to look at many truncates and related proteins. Selectivity was a key a factor in this project and series were selected for progression based on this criteria. GCI is able to predict if the fragments bind in a 1:1 or heterogeneous manner aiding decision making. Binders recorded by GCI were validated using thermal shift data (nanoDSF) to gain confidence in binding and triage compounds for crystallography selection.



Structural Biology

Several crystal structures with fragments bound were collected showing binding. One structure in particular was of interest due to vectors presented from the fragment hit and the features of the binding site. The binding site has a potentially exploitable motif to improve selectivity. Plans to expand on this hit are in place.

Services/Contact

If you would like to learn more about applying our drug-discovery platforms, please contact: enquiries@domainex.co.uk or Nick.Martin@Domainex.co.uk
Scan here for more information on the FragmentBuilder platform:

References:

Whitepaper: The Throughput Booster for Binding Interaction Screening – the waveRAPID Kinetics Assay; Creoptix AG