P05 Fragment library screening by Grating Coupling Interferometry (GCI) and benefits over Surface Plasmon Resonance (SPR)

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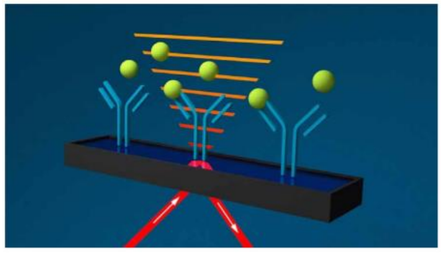
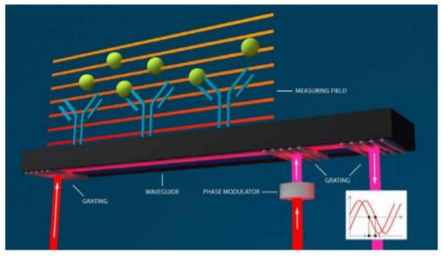
1 Grating-coupled interferometry (GCI)

- Surface-based, label-free biosensing technique.
- Target molecules (e.g. proteins) are immobilized to the sensor surface,
- Waveguide runs parallel to the chip surface
- 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.
- Longer length of the waveguide compared to other chip-based methods provides intrinsically high signal-to-noise levels for improved sensitivity.
- Ability to resolve extremely rapid dissociating kinetics, and innate compatibility with the high molecular weight ratios,
- Improves fragment-based screening and kinetic analysis of small molecules to accelerate drug development compared to other chip-based methods.



Traditional Surface Plasmon Resonance

- The ligand of interest is immobilised.
- Molecular interactions are detected as changes in refractive index within an evanescent field (orange) of the surface plasmon shown as energy dips at specific incidence angle.

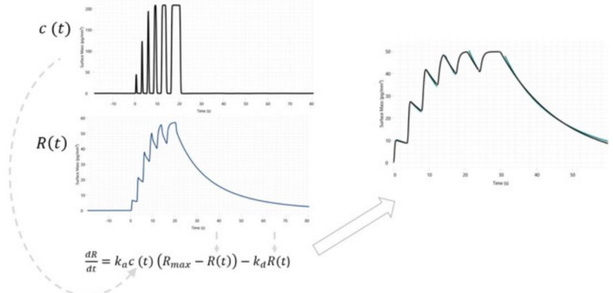


Grating-coupled interferometry (GCI)

- Waveguide runs parallel to chip surface increasing length of waveguide Reference beam is also coupled into the waveguide. Consequently, interference happens within the waveguide
- Hhigh-resolution, time-dependent and robust phase shift signal is created .

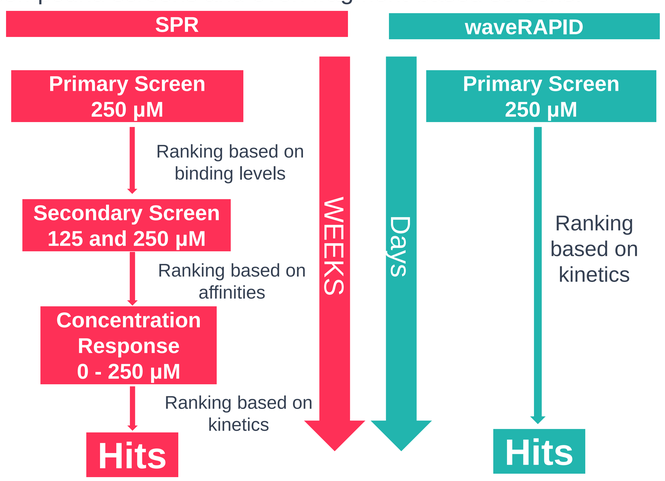
2 waveRAPID stands for Repeated Analyte Pulses of Increasing Duration.

- Single analyte concentration pulse applied for increasing duration
- Observed binding curve is a response to the time-dependent concentration input of the injected analyte.
- waveRAPID uses varying injection durations from a single well to achieve the same result in a fraction of the time – a robust determination of kinetic parameters.
- Reduces screening and compound preparation time



3) Screening: waveRAPID technology accelerates the capture of kinetic measurements.

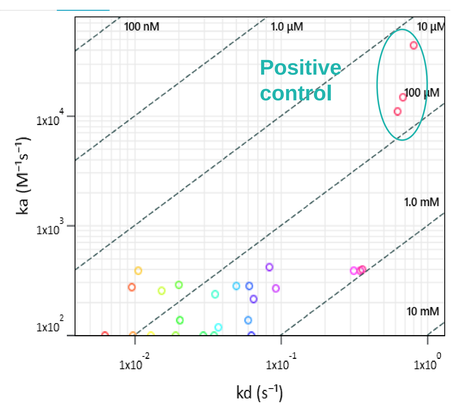
- Kinetic parameters determined for analytes during the primary screen. Follow up assays usually performed with traditional kinetic are no longer required.
- Shortened assay time allows screening of inherently unstable species more easily.
- Ability to detect weak binding interactions with fast off-rates (up to $10s^{-1}$) more reliably it is optimally suited for small molecule compound screens and even fragment-based screens.



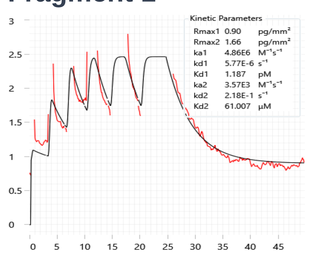
4) Fragment Screen of E3 Ligase target:

- 1100 fragments from the Domainex Fragment Library were screened in RAPID mode against the full length protein (120 kDa) at 250 µM.
- Complete screen was completed in 4x 15h experimental runs.
- Recorded kinetic data was filtered by association error, dissociation error, R_{max} and K_D to identify fragment hits. The k_a/k_d plot of one of the runs shows very good reproducibility of the positive control and the distribution of fragment hits.
- Total of 30 hits could be identified.

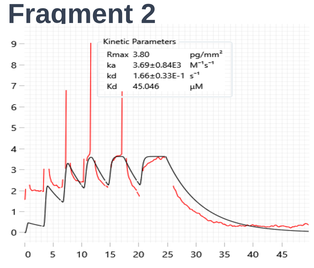
waveRAPID k_a/k_d plot



Fragment 1



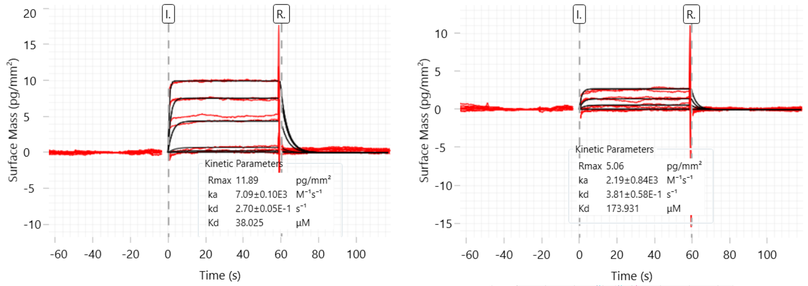
Fragment 2



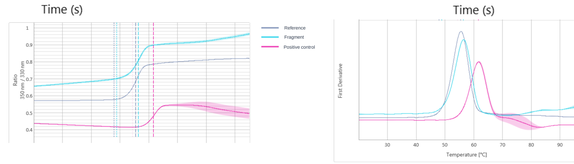
5) Counter screening and hit validation:

- Fragment hits from primary screening were counter screened against the substrate binding domain of the E3 ligase to triage fragments binding to the substrate domain or ubiquitin binding domain.
- Comparable kinetic parameters were identified for six fragments against the full length and substrate binding domain.
- Traditional kinetic measurements confirmed data obtained using RADIP mode validating the new screening methodology.
- All six fragments also showed binding in a thermal shift assay using nanoDSF.

Traditional Kinetics



nanoDSF



6) Summary:

Domainex has invested in the Creoptix wave Delta instrument. Here we have shown that using the new waveRAPID technology significantly shortens screening times compared to traditional SPR while maintaining excellent data resolution even at large ligand:analyte MW ratios.

Domainex welcomes interest from any potential collaborators, industrial or academic. If you would like to learn more about applying our drug-discovery platform to other targets, please contact:

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References:

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

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