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Fragment-Based Screening

Creoptix™ WAVE

Creoptix AG, Einsiedlerstrasse 34, CH-8820 Wädenswil, Switzerland, www.creoptix.com, info@creoptix.com

Summary

Fragment-based drug screening has emerged as a promising tool for the discovery of new chemical entities, especially for targets that prove to be unamenable to classical high-throughput screening (HTS). Several label-free interaction analysis methods exist but mainly NMR, X-ray crystallography and surface plasmon resonance (SPR) have been applied for fragment-based screening. The fragments' low molecular weight and their rather weak affinity but also limited availability of the target protein and unstable targets, ask for methods with increased sensitivity at high reproducibility and reliability. Here, we demonstrate that with **Creoptix™ WAVE**, a novel waveguide-based interferometry device, extremely low standard deviations can be achieved, making this system especially useful for targets that are unstable or otherwise of low activity.

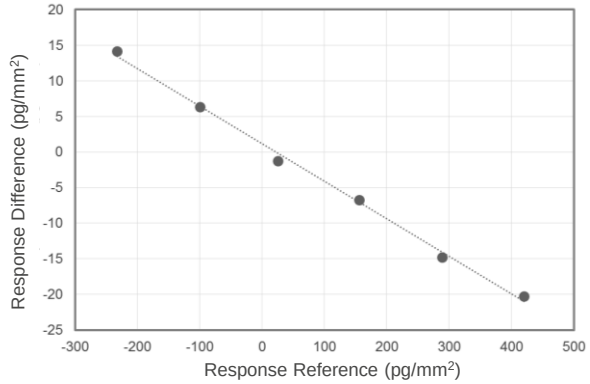

Figure 1: *Solvent correction curve for HBS-EP buffer with 1.5-2.75% DMSO in steps of 0.25%.*

Results & Conclusion

A library of 531 fragments was screened against a 25 kDa target protein (fragments and protein kindly provided by Roche, Switzerland). First, target protein was amine-coupled on polycarboxylate WAVEchips at density levels of 3'000 and 5'700 pg/mm^2, respectively. For solvent correction, a DMSO correction curve with HBS-EP buffer containing 1.5-2.75% DMSO was then established (Fig. 1). The linear response for increasing DMSO concentrations proved to provide high confidence in solvent correction.

The 531 fragments with molecular weights of 84 to 339 Da (average 208 Da) were then tested against the target protein at a concentration of 0.1 mM derived by a fifty-fold dilution of DMSO-stocks with HBS-EP (Fig. 2).

As positive control a 455 Da reference compound was injected after every 10th fragment as well as a blank. Standard deviations to the trendline were only 0.194 pg/mm^2 for the positive and 0.267 pg/mm^2 for the negative control, allowing for an excellent window of confidence even at decreasing binding activity of the ligand protein over time.

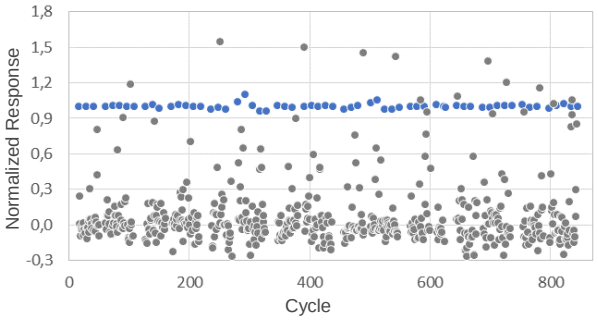

Figure 2: *Reference–subtracted responses for 531 fragments (84 to 339 Da size range with average molecular weight of 208 Da) after solvent correction, molecular weight adjustment and normalization of ligand activity. Reference compound is schown in blue.*

Finally, to verify data reproducibility, a same sub-set of fragments was tested in two independent runs (Fig.3). The correlation of the normalized responses with a correlation coefficient of r^2=0.9761 demonstrates excellent reproducibitlity.

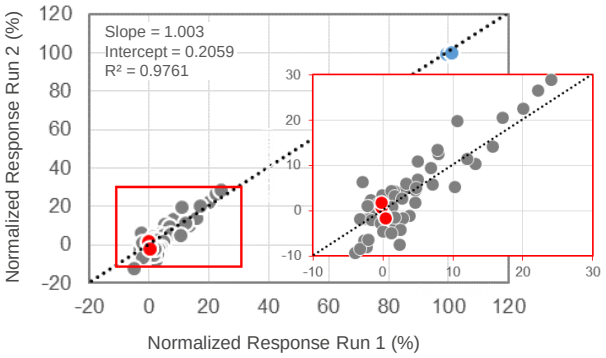

Figure 3: *Normalized responses of two independent runs with a same sub-set of fragments (fragments in grey, positive control in blue and blank injections in red). The red-framed area of the graph is shown enlarged in the insert*

In conclusion, the **Creoptix™ WAVE** system provides a new tool for fragment-based screening with excellent reproducibility. Thanks to its outstanding sensitivity, even low signal levels can be resolved with high confidence, making this system especially useful for targets that are large or unstable.

For further information about **Creoptix™ WAVE**, visit us on www.creoptix.com.