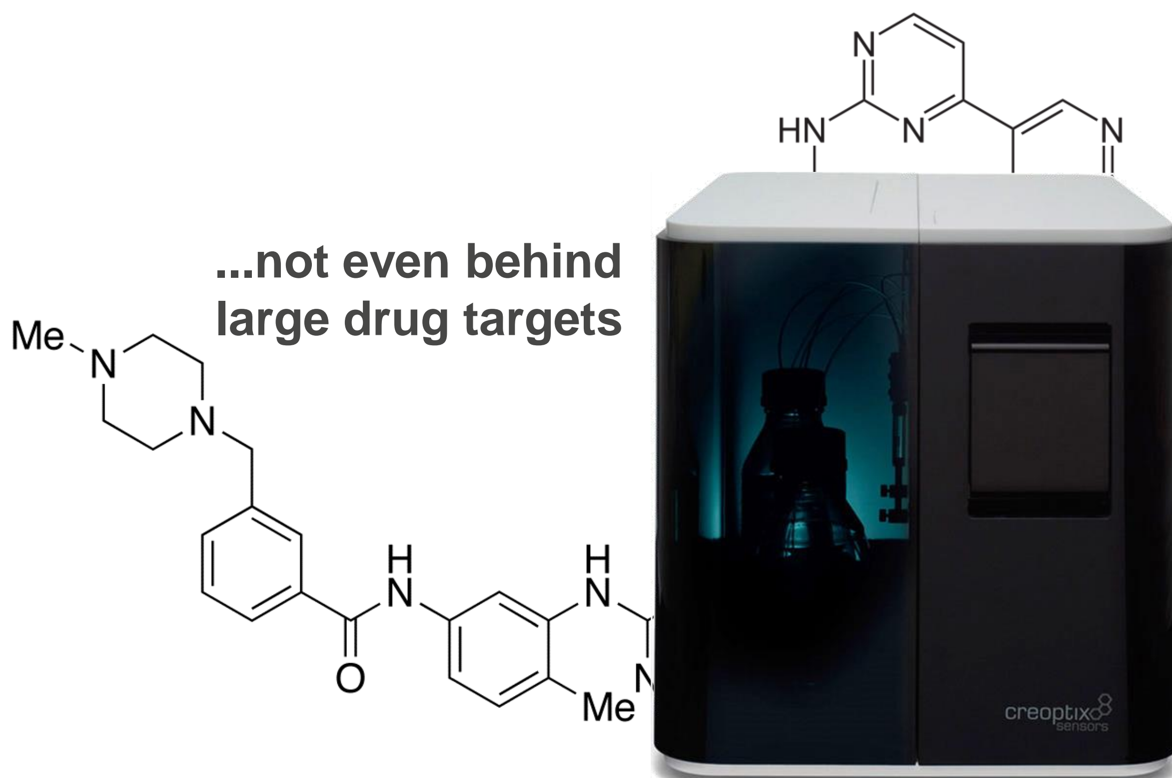
creoptix sensors

Tech Note 08 / March 31, 2016

Small Molecules can‘t hide anymore



Sensitivity is key and often limiting for accurate and reliable analysis of molecular interactions. This is especially true for large drug targets in combination with small molecule inhibitors. Here we demonstrate the power of the exceptional sensitivity of the waveguide interferometry based Creoptix WAVE system when analysing the interaction of a small molecule of 297 Da with a large drug target of 110 kDa. Traditionally an interaction of such large size ratio of drug target to analyte could only be reliably measured by maximizing the Rmax, therefore saturating the surface with the target. Thanks to the outstanding resolution of the Creoptix WAVE, reliable kinetics was recorded for this target-analyte pair within a broad range of immobilization levels and thus responses, showing that the effective limit for the target to analyte molecular weight ratio can now be pushed up to >1000:1.

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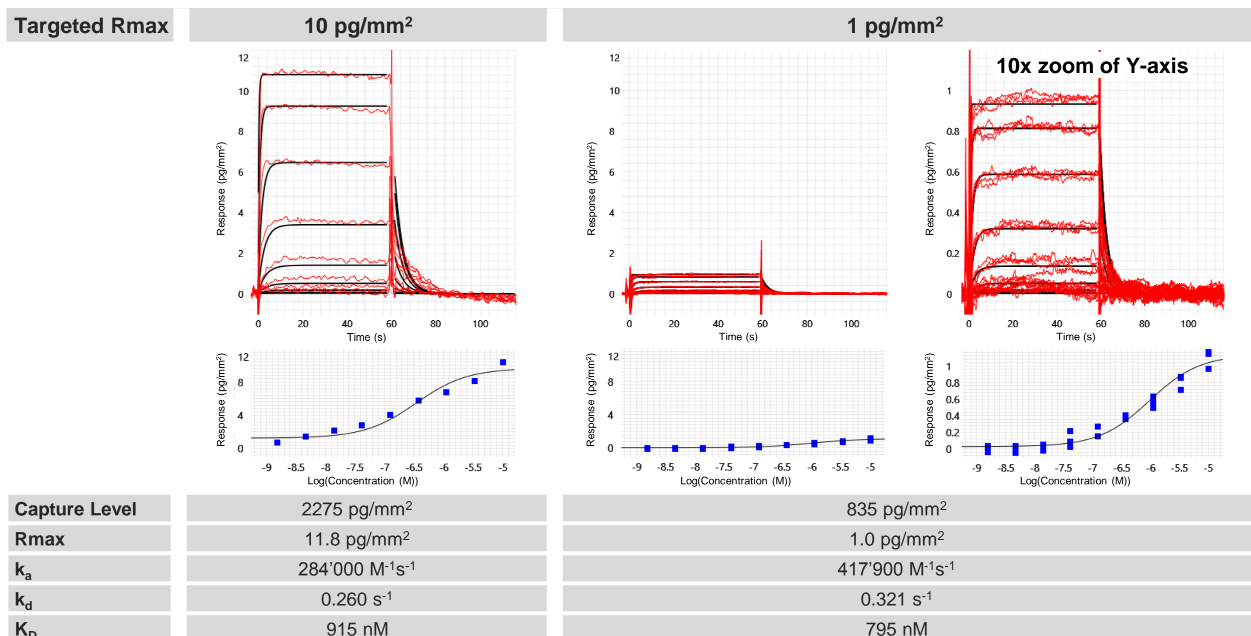
Experiment

Target (undisclosed protein, 110kDa, biotinylated, present as tetramers) and Analyte (undisclosed small molecule inhibitor, 297 Da) with expected affinity below 1 µM were kindly provided by Novartis (NIBR, Cambridge MA, USA). Throughout all experiments running buffer was 20mM Hepes, 300mM NaCl, 1mM DTT, 2%DMSO at pH 7.5 (all reagents from Sigma). High capacity Creoptix WAVEchip PCH-S (surface with Streptavidin coating) were used to capture the Target with a flow of 10µl/min to the desired surface density level. The kinetic run (all at 80µl/min) included 15-30 startup cycles of running buffer injections, followed by a 1:3 dilution series of the Analyte (9 concentrations, with 10µM as highest concentration, in triplicates injected in reverse orders) with one blank every 3 injections. DMSO calibrations were performed at the end of the series. All the cycles in the kinetic run included 45s baseline, 60s association and 60s dissociation. No regeneration was required.

Data adjustments and analysis were performed with the Creoptix WAVEcontrol software. Adjustments included DMSO calibration correction, X-offset correction and blank subtraction using closest blank. Global fitting with bulk correction for both association and dissociation was used to obtain the kinetic rate constants k_a and k_d, as well as the Rmax. Equilibrium analysis included an offset correction.



Targeted Rmax	10 pg/mm²	1 pg/mm²
Capture Level	2275 pg/mm²	835 pg/mm²
Rmax	11.8 pg/mm²	1.0 pg/mm²
k_a	284'000 $M^{-1}s^{-1}$	417'900 $M^{-1}s^{-1}$
k_d	0.260 s^{-1}	0.321 s^{-1}
K_D	915 nM	795 nM

Conclusion

Excellent kinetic data fits were obtained for the interaction between a very big Target protein (110kDa) and a small molecule Analyte (297Da) at different Rmax. Such Target to Analyte molecular weight ratios of >350:1 present a big challenge for traditional label-free interaction analysis systems such as SPR (Surface Plasmon Resonance) as they exceed, or are very close to, the limit of what can be measured. Therefore saturating the surface with the ligand to maximize the Rmax is required, albeit at a significant cost in data quality. On the Creoptix WAVE in contrast, this interaction could be reliably measured at different ligand densities well below surface saturation. In addition, the presented data has a 10-fold dynamic range in terms of Rmax. We can therefore conclude that the kinetics of a system exhibiting a molecular size ratio of >1000:1 can now be reliably determined on the Creoptix WAVE system for molecular interaction analysis.