

# Highly sensitive analysis at low immobilization levels, without mass transport limitation

Creoptix® WAVEsystem



Kinetics below  $R_{\max} < 1$

In this TechNote we show the remarkable sensitivity of the WAVEsystem. Thanks to the expanded sensing field over which the Grating-Coupled Interferometry (GCI) technology measures, high-resolution kinetic determination at even very low responses is possible, potentially reducing material costs significantly

## Summary

During label-free analysis of molecular interactions, mass transport limitation represents a significant source of deviation from first-order kinetics. Arising from analyte concentration gradients between the bulk solution and the surface, mass transport limitation is especially pronounced at the high-density ligand immobilization levels often required to generate a measurable binding signal.

Using a proprietary Grating-Coupled Interferometry (GCI) technology, the Creoptix® WAVEsystem delivers superior sensitivity over traditional Surface Plasmon Resonance (SPR) methodologies, particularly with low ligand immobilization levels of poorly active proteins. This translates to reliable kinetics at even very responses ( $R_{max} < 1$ ).

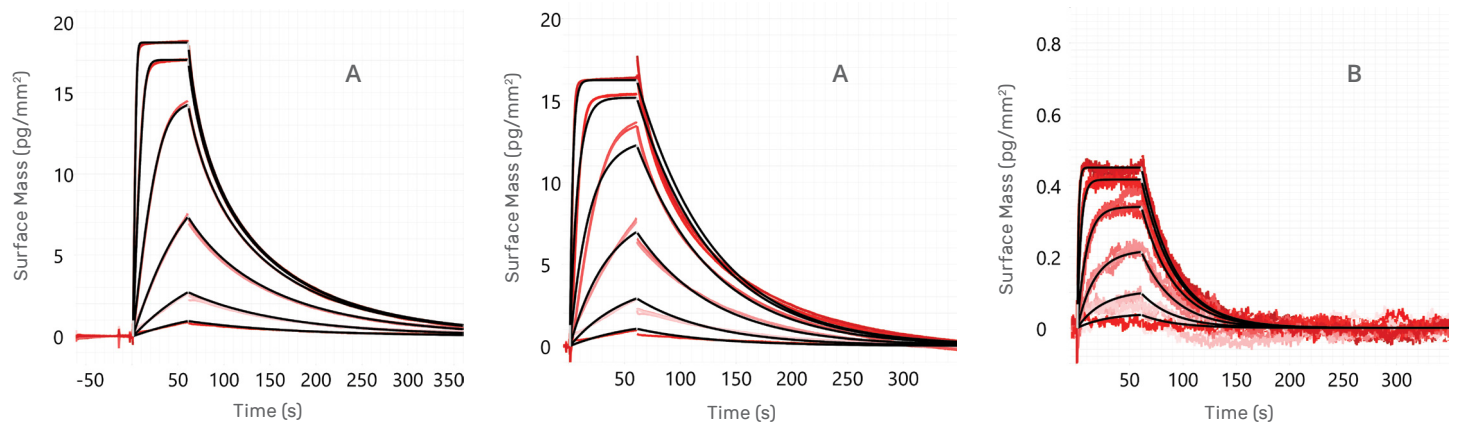
By characterizing the interaction between acetazolamide and Carbonic Anhydrase II, using different immobilization densities of the enzyme, we show that the Creoptix WAVEsystem affords greatly reduced sample consumption while avoiding mass transport limitation.

**Table 1: Kinetic data for acetazolamide binding onto different Carbonic Anhydrase II (CAII) densities**

	CAII capture level (pg/mm <sup>2</sup> )	$k_{on}$ (M <sup>-1</sup> ·s <sup>-1</sup> )	$k_{off}$ (s <sup>-1</sup> )	$k_m$	$R_{max}$ (pg/mm <sup>2</sup> )	$K_D$ (nM)
Kinetic data MTL model	8100	$1.29 \times 10^6$	0.0418	$6.12 \times 10^6$	18.688	32.5
Kinetic data 1:1 model	1240	$0.77 \times 10^6$	0.0324	-	0.469	42.3

Legend: (A) Sensorgrams showing the interaction between acetazolamide (analyte, MW 222.25 Da) and carbonic anhydrase II (CAII, ligand, MW 29 kDa) at low and high immobilization densities. CAII was captured on a 4PCH WAVEchip at different immobilization densities, as indicated. Dose-response curves for acetazolamide were recorded at each immobilization level. Mass transport limitation (MTL) model was used to fit the data from the high immobilisation level (left) as the interaction deviated from 1:1 model (right) using Creoptix WAVEcontrol software. (B) The data shows that the kinetic fits at the low immobilization level are not mass-transport limited, but rather follow first-order kinetics.

**Figure 1: Acetazolamide binding onto different Carbonic Anhydrase II (CAII) densities**



\* 1 pg/mm<sup>2</sup> = 1 RU

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## Keeping Kinetics Real



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