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Avoiding Mass Transport

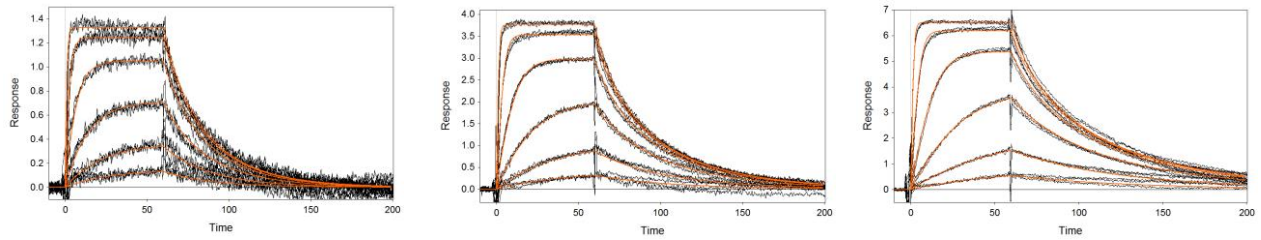
Creoptix™ WAVE

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

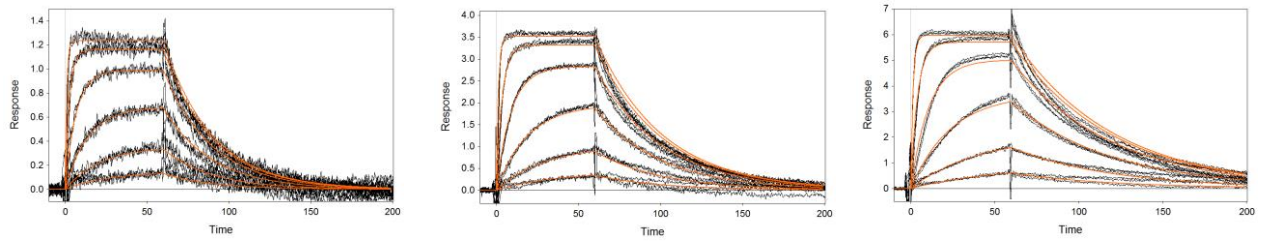
Summary

In surface-based label-free interaction analysis, a main source for deviation from first-order kinetics is mass transport limitation derived from analyte concentration gradients between the bulk and the surface. This effect is particularly seen with fast on-rates and high density ligand immobilization levels required to generate sufficiently strong binding signals.

Here we show that the exceptionally high sensitivity of the **Creoptix™ WAVE** system enables accurate and reliable kinetics at very low ligand immobilization levels thereby not only reducing sample consumption but especially avoiding mass transport limitation.



Kinetics with mass transport limitation					
bCAII capture level (pg/mm^2)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	k_m	R_{max}	K_D (nM)
231	$1.40(1)\times10^6$	0.0482(4)	$4.2(1)\times10^6$	1.377(3)	34.6(1)
467	$1.485(7)\times10^6$	0.0509(2)	$4.00(2)\times10^6$	3.894(3)	34.27(6)
1472	$1.414(6)\times10^6$	0.0386(2)	$5.36(2)\times10^6$	6.687(5)	27.30(4)



Kinetics without mass transport limitation					
bCAII capture level (pg/mm^2)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	k_m	R_{max}	K_D (nM)
231	1.056×10^6	0.03382	-	1.276	32.041
467	7.806×10^5	0.02481	-	3.635	31.780
1472	6.848×10^5	0.01682	-	6.119	24.558

Legend: *Sensorgrams of the interaction between Acetazolamide (222.25 Da) as analyte and Carbonic Anhydrase II (CAII, 29 kDa) as ligand at increasing immobilization densities on the same surface: Minimally biotinylated CAII was captured on a streptavidin pre-coated sensor (WAVEchip STH) at increasing immobilization densities of 231, 467 and 1472 pg/mm^2, respectively. Dose response curves of Acteazolamide were generated after each immobilization level and the kinetics were fitted with (top) or with mass transport limitation (bottom) by Scrubber software. The date show that kinetics fits at low immobilization level are not mass transport limited, leading to first-order kinetics.*