

# REAL SAMPLES, REAL DATA: MOLECULAR INTERACTION ANALYSIS BY GRATING-COUPLED INTERFEROMETRY



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## A NEW LEVEL OF PERFORMANCE AND FLEXIBILITY IN DRUG DISCOVERY

By bringing together modern label-free technology, application development know-how and sophisticated software, Creoptix offers a unique optical biosensor tool for binding kinetics. Engineered around our proprietary Grating-Coupled Interferometry (GCI)<sup>1</sup> technology, the Creoptix WAVEsystem delivers high-quality kinetic data across a broader range of samples than traditional SPR equipment.

Assess drug performance in undiluted biofluids for reliable kinetic profiling in conditions closer to real life

Screen, rank and characterize weak binders with off-rates up to  $10\text{ s}^{-1}$

Quantify binding affinities ( $K_D$ ) from low pM to high  $\mu\text{M}$  with confident kinetic analysis

## GRATING-COUPLED INTERFEROMETRY (GCI)



GCI is a surface-based, label-free biosensing technique. When target molecules (e.g. proteins) are attached to the sensor surface, binding of analytes leads to an increase in mass and hence to a change in the refractive index within the evanescent field near the surface. In GCI, refractive index changes on a sensor surface are measured as time-dependent phase-shift signals. The long light-to-sample interaction length of the waveguide provides intrinsically high signal-to-noise levels for improved sensitivity.

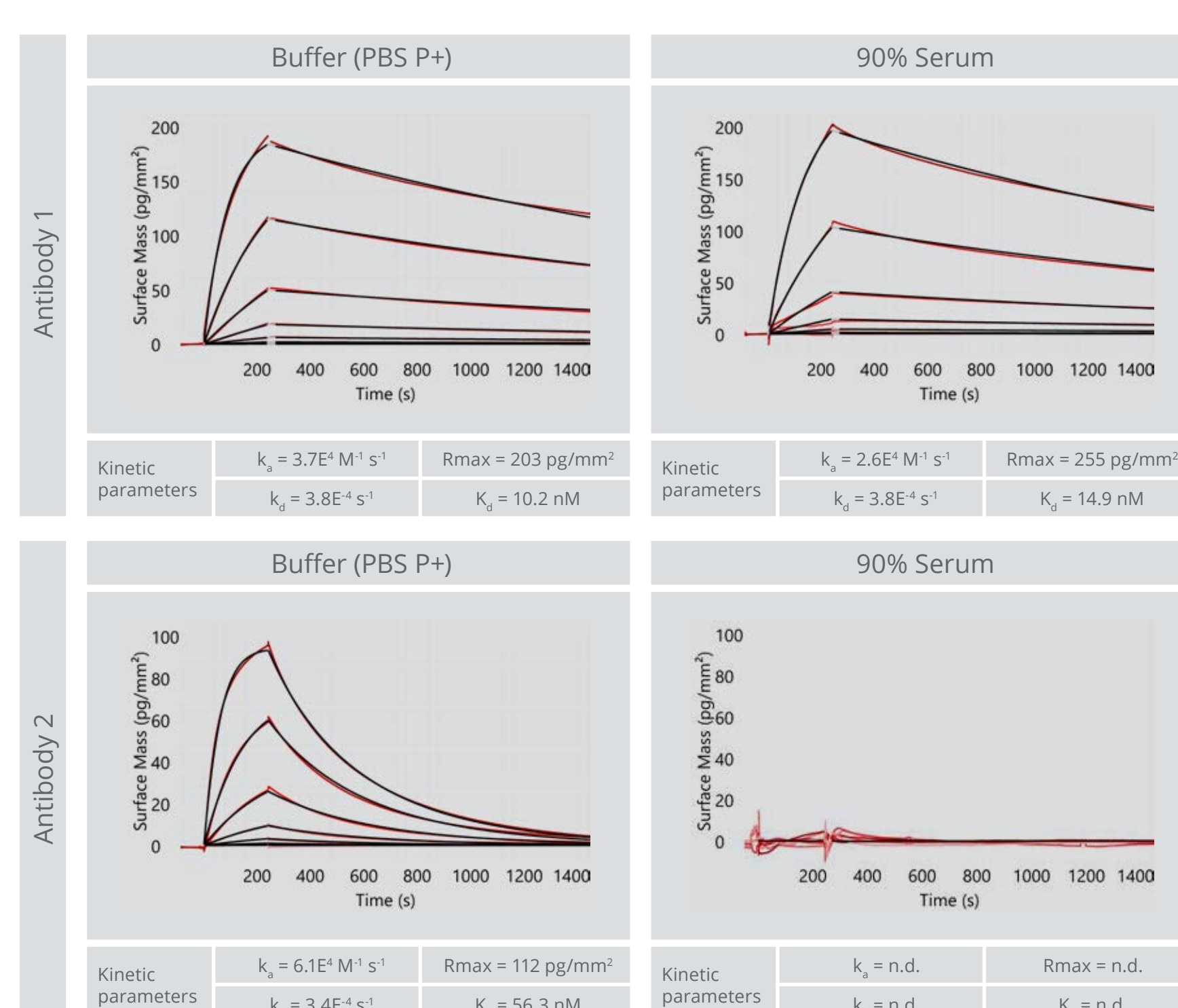
The Creoptix<sup>®</sup> WAVEsystem combines GCI with innovative no-clog microfluidics, allowing the study of interactions even between very small ligands and large complexes. The system uses a robust microfluidic sensor, the WAVEchip<sup>®</sup>, where (membrane) proteins,<sup>2</sup> antibodies, VLPs, peptides or other molecules can be immobilized using various chemistries.



### ANTIBODY PROFILING IN BIOFLUIDS

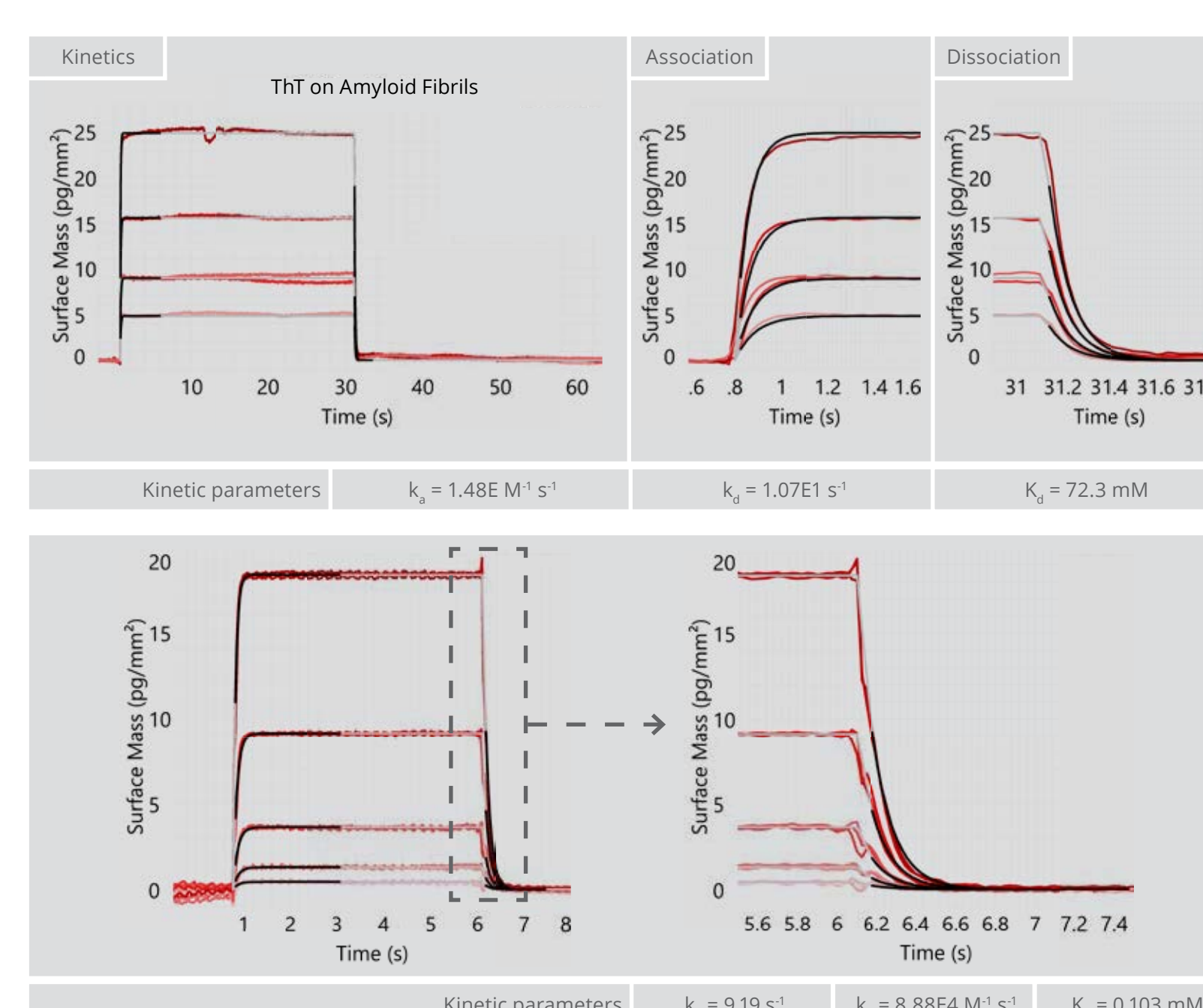
The robust sensor and microfluidics of the WAVEsystem allows the kinetic characterization of molecular interactions in almost pure serum or plasma. Binding proteins developed for diagnostic applications can therefore be directly profiled in great detail within the respective crude matrix.

Here, a full kinetic interaction analysis acquired in the course of an antibody profiling study is shown. Two different antibodies were immobilized on a 4PCP WAVEchip via amine coupling. The respective antigen was injected in either buffer (PBS P+) or 90% human serum in a dilution series of eight (8) concentrations ranging from 137 pM to 300 nM at 95  $\mu\text{l}/\text{min}$  for 240s followed by 1200s dissociation. Raw data were double referenced and globally fit with a 1:1 binding model. For antibody 2 no binding was observed in serum.



### WEAK BINDERS, STRONG DATA

Weak binders such as those found in fragment-based screening are typically ranked by affinity rather than kinetics due to their very fast off-rates, which can not be resolved by traditional SPR instrumentation. Here we show that the Creoptix<sup>®</sup> WAVEsystem provides an outstanding resolution whereby very fast kinetics can be reliably determined at off-rates up to  $10\text{ s}^{-1}$ .

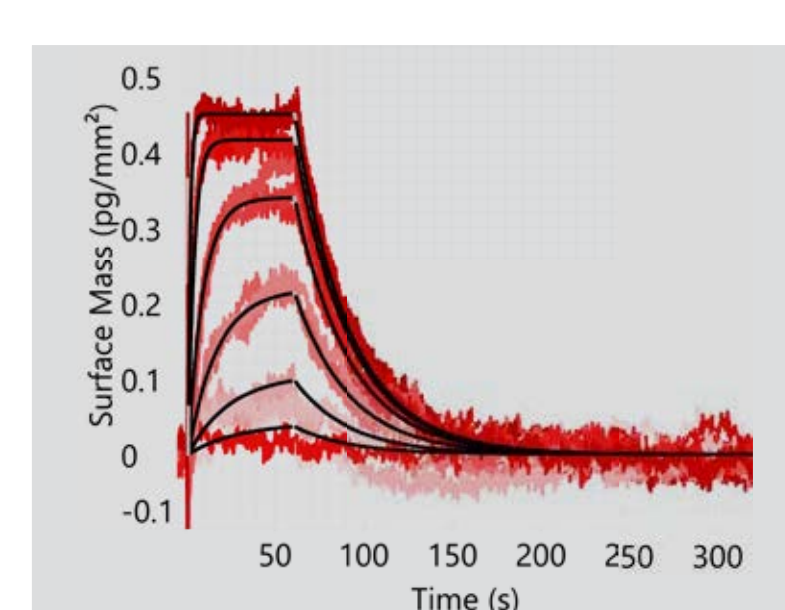


Self-assembled amyloid fibrils were immobilized via amine coupling on a 4PCP WAVEchip<sup>®</sup> (zwitterionic surface). The small molecule thioflavin (THT, 319 Da) was injected in four (4) concentrations (50 nM - 6.25 nM) for 30s at 400 ml/min. Raw data were double referenced and globally fit with a 1:1 binding model showing accurate determination of an off-rate around  $10\text{ s}^{-1}$ .

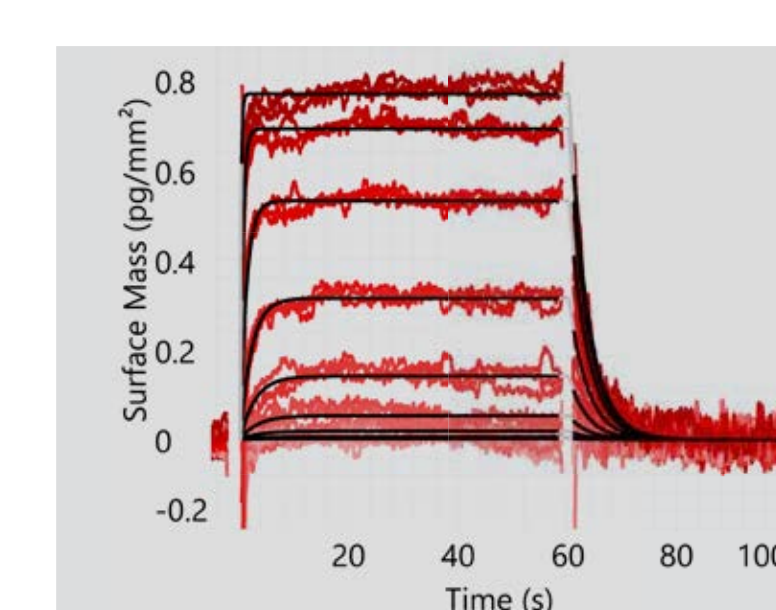
Sensorgrams of a 6-mer oligonucleotide (1.7 kDa) binding onto its complementary ssDNA (11 kDa biotinylated 34-mer) captured on streptavidin on a PCP-5 WAVEchip. The interaction was measured at 25°C. Zoom into the dissociation shows excellent data fitting and an accurate determination of an off-rate around  $10\text{ s}^{-1}$ .

### SMALL MOLECULES CAN'T HIDE ANYMORE

Sensitivity is key and often limiting for accurate and reliable analysis of molecular interactions. The high-sensitivity of the Creoptix<sup>®</sup> WAVEsystem allows researchers to confidently analyze binding interactions at very low signal levels and high analyte-to-ligand molecular weight (MW) ratios.



Sensorgrams of acetazolamide (222 Da) binding to Carbonic Anhydrase II (29 kDa) immobilised at low density onto a PCH WAVEchip.



Sensorgrams of a small drug molecule (295 Da) binding to a target protein (110 kDa) immobilised at low density onto a PCH WAVEchip. Note the analyte-to-ligand MW ratio is > 300.

GCI IS FEATURED IN



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