MOLECULAR INTERACTION ANALYSIS BY GRATING-COUPLED INTERFEROMETRY (GCI)

Matyas Vegh[1], Jealemy Galindo[1], Fabio Andres[1], Rony Nehme[1], Fabio M. Spiga[1], Marten Beeg[2]

1 Creoptix AG, 8820 Wädenswil, Switzerland, info@creoptix.com

2 Istituto di Ricerche Farmacologiche Mario Negri IRCCS, 20156 Milano, Italy, marten.beeg@marionegri.it

ABSTRACT

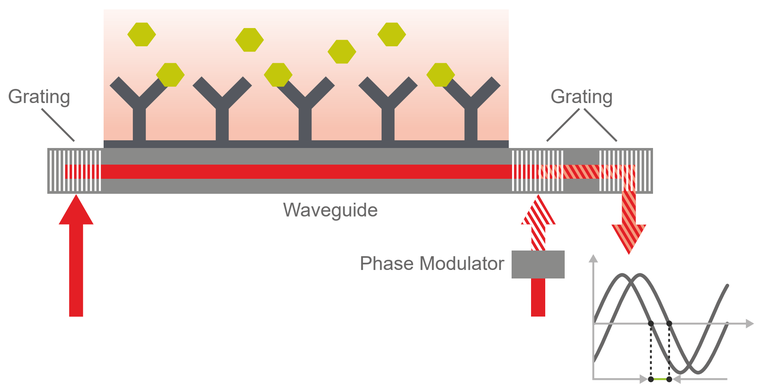
Protein interaction analysis is crucial for the successful identification of drug targets. Amongst the wide range of biophysical techniques available today, surface-based techniques such as SPR utilize optical systems to study biomolecular interactions without labels.

Grating-Coupled Interferometry (GCI)[1] is a novel label-free technique suitable for the analysis of low-molecular-mass drugs, multiprotein complexes and even larger particles like viruses or bacteria, with a remarkably wide affinity range (from mM to pM).[2]

Here, we show selected examples of GCI applications in the field of antibody characterization, including binding kinetics and affinity measurements in phisiologically relevant media (serum, plasma, cell supernatant), that highlight how this technology is revolutionizing the study of molecular interactions and drug discovery.

GRATING-COUPLED INTERFEROMETRY (GCI)

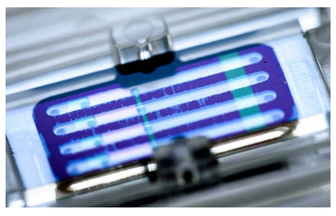
GCI is a surface-based, label-free biosensing technique. When target molecules (e.g. proteins Y) are attached to the sensor surface, binding of analytes (⬢) leads to an increase in mass and hence to a change in the refractive index within the evanescent field near the surface.



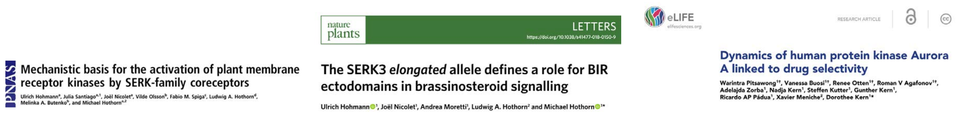
In GCI, refractive index changes on a sensor surface are measured as time-dependent phase-shift signals. The long-light to sample interaction length of the waveguide provides intrinsically high signal-to-noise levels.

The Creoptix® WAVE system combines GCI with innovative no-clog microfluidics, allowing the study of interactions even between very small ligands and large receptors.

The system uses a robust sensor and microfluidics cartridge, the WAVEchip®, where proteins, such as antibodies, VLPs, peptides or other molecules can be immobilized using various chemistries.



GCI IS FEATURED IN



REFERENCES

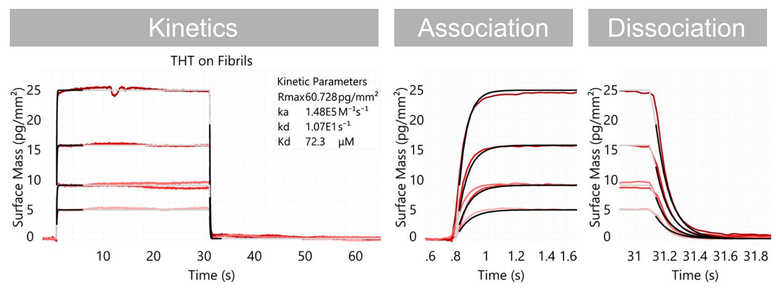
1_P. Kozma et al., “Grating coupled optical waveguide interferometer for label-free biosensing”, Sensors and Actuators B: Chemical, 155:446–450 (2011)

2_Hohmann et al., "The SERK3 elongated allele defines a role for BIR ectodomains in brassinosteroid signalling.", Nature Plants, 4:345–351 (2018)

KINETICS ON FIBRILS

The WAVE system allows for binding interaction analysis of small molecules onto fibrils.

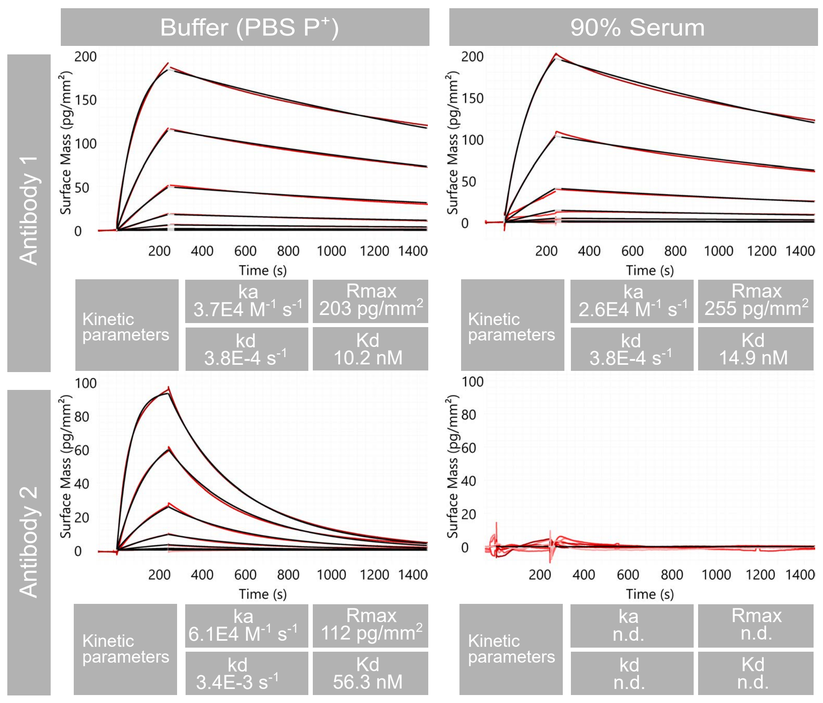
Self-assembled fibrils were immobilized via amine coupling on a 4PCZ WAVEchip® (zwitterionic surface) at a surface density of around 8000 pg/mm^2. The small molecule thioflavin (THT, 319 Da) was injected in four (4) concentrations (50 μM - 6.25 μM) for 30s at 400 μl/min. Raw data were double referenced and globally fit with a 1:1 binding model.



ANTIBODY PROFILING IN SERUM

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

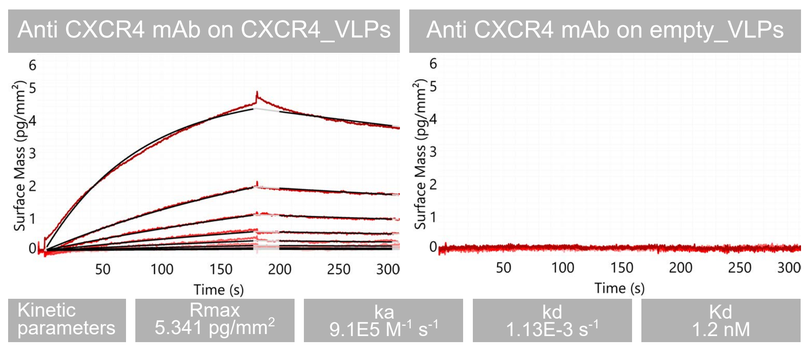
Here, a full kinetic interaction analysis acquired in the course of an antibody profiling study is shown. Two different antibodies were immobilized on a 4PCP chip via amine coupling. The respective antigen was injected in either buffer (PBS P+) or 90% human serum in a dilution series of eight (8) concentrations ranging from 137 pM to 300 nM at 95 μl/min for 240s followed by 1200s dissociation. Raw data were double referenced and globally fit with a 1:1 binding model. For antibody 2 in serum fitting was not possible, demonstrating the absence of any constructive interaction in the crude matrix.



KINETIC ANALYSIS ON MEMBRANE PROTEINS

The WAVE system allows for the kinetic characterization of analytes binding to membrane proteins (GPCR) embedded in VLPs with minimal preparation (one-step centrifugation).

A 4PCP WAVEchip® was coated with Wheat Germ Agglutinin prior to injecting CXCR4_VLP and empty_VLPs (from Integral Molecular). Twelve (12) concentrations of the mAB anti-CXCR4 (ranging from 24 pM to 100 nM) were injected for 180s at 60 μl/min. Raw data were double referenced and globally fit with a 1:1 binding model.



KINETICS ON GPCRs FROM CRUDE MEMBRANES

The WAVE system allows for the kinetic characterization of analytes binding to membrane proteins (GPCRs) captured from crude cell membrane extracts (centrifugation-sonication).

CHO cells crude membrane extracts (from Novartis) expressing: the Nt-AVI target GPCR (red), the Ct-AVI target GPCR (green) and the negative control Nt-AVI GPCR (blue) were injected onto a 4PCP-STA WAVEchip® at 10 μl/min. Subsequently, five (5) concentrations (0.96 nM - 3 μM) of the anti-target-GPCR nanobody (scAb) were injected in duplicates for 180s at 45 μl/min.