Antibody Characterization in Physiologically Relevant Conditions by Grating-Coupled Interferometry (GCI)

Matyas Vegh[1], Jealemy Galindo[1], Fabio Andres[1], Fabio M. Spiga[1], Richard J. Isaacs[2]

1 Creoptix AG, 8820 Wädenswil, Switzerland, info@creoptix.com
2 Creoptix, Inc, 111 South Bedford Street, Ste.108, Boston, MA-01803, Burlington, USA

Abstract

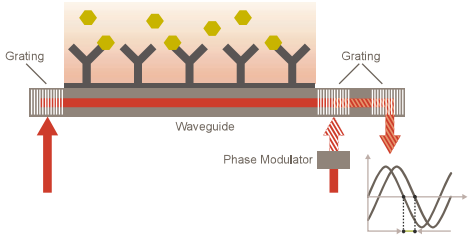
Protein interaction analysis is crucial for the successful identification of drug targets. Amongst the wide range of biophysical techniques available today, surface-based biosensors such as SPR utilize optical systems to study biomolecular interactions without labels.

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 physiologically 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) technology

GCI is a surface-based, label-free biosensing technique. When target molecules (e.g. proteins Y) are attached to the sensor surface, binding analytes ( ) lead 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[3].

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

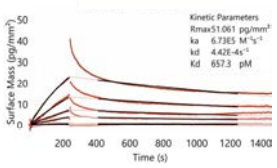
1_P. Kozma et al., "Grating coupled optical waveguide interferometer for label-free biosensing", Sensors and Actuators B: Chemical, 155:446–450 (2011)
2_Hohmann et al., "The SERK3 elongated allele defines a role for BIR ectodomains in brassinosteroid signalling,", Nature Plants, 4:345–351 (2018)
3_www.sprpages.nl/how-low

Kinetics in Serum and Plasma

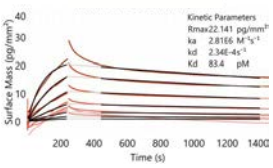
Binding interaction analysis in physiologically relevant conditions is possible using GCI.

Monoclonal antibodies (mAbs) were immobilized via amine coupling on a 4PCH chip. The protein analyte was spiked in 90% serum or 90% plasma as indicated, and injected in six concentrations (4 nM - 31 pM). Raw data were double referenced and globally fit with a 1:1 binding model.

Kinetics in 90% bovine serum



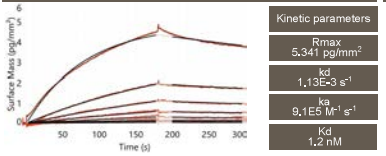
Kinetics in 90% human plasma



Kinetic Analysis on GPCRs

GCI can be used to kinetically characterize molecules binding to G-Protein-Coupled Receptors (GPCRs) directly from crude membrane preps or virus-like particles (VLPs).

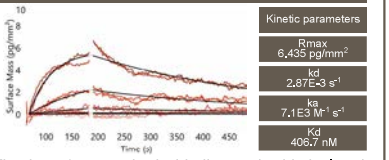
Anti CXCR4 mAb on CXCR4-VLPs



Kinetic parameters	
Rmax	5,341 pg/mm²
kd	1,13E-3 s⁻¹
ka	9,1E5 M⁻¹ s⁻¹
Kd	1.2 nM

CXCR4-VLPs (Integral Molecular) were captured on a 4PCP chip coated with Wheat Germ Agglutinin (WGA). Kinetics of an anti-CXCR4 mAb interacting with CXCR4-VLPs were determined by injecting twelve concentrations (24 pM - 100 nM) of the mAb.

Nanobody kinetics on GPCR in crude membrane preparation



Kinetic parameters	
Rmax	6,435 pg/mm²
kd	2,87E-3 s⁻¹
ka	7,1E3 M⁻¹ s⁻¹
Kd	406.7 nM

Kinetics of a nanobody binding to its biotinylated GPCR target in a crude membrane prep (captured on a PCP-STA chip) were determined by injecting five concentrations of the nanobody (0.96 nM to 3 μM).

Antibody Profiling in Serum

The robust sensor and microfluidics of the technology 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 depth within the relevant matrix.

Comprehensive interaction data directly acquired in early-stage antibody profiling enables valuable kinetic characterization of diagnostic leads in the physiologically relevant matrix. Here, two different antibodies were amine-coupled to a 4PCP chip and the antigen was injected in either buffer (PBS P+) or 90% human serum in a dilution series of eight concentrations (137 pM to 300 nM). Raw data were double referenced and globally fit with a 1:1 binding model.

Antibody 1 demonstrates its suitability for diagnostic application, revealing highly similar kinetics in 90% serum as in buffer. On the other hand, antibody 2 evidently shows no binding activity in the presence of serum and is therefore not suited for diagnostic application.

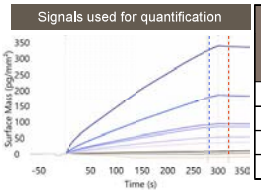
	Buffer (PBS P+)		90% Serum	
Antibody 1	Kinetic parameters	ka 3,7E4 M⁻¹ s⁻¹	Kinetic parameters	ka 2,6E4 M⁻¹ s⁻¹
		Rmax 203 pg/mm²		Rmax 256 pg/mm²
		kd 3,8E-4 s⁻¹		kd 3,8E-4 s⁻¹
		Kd 10,2 nM		Kd 14,9 nM
Antibody 2	Kinetic parameters	ka 6,1E4 M⁻¹ s⁻¹	Kinetic parameters	ka n.d.
		Rmax 112 pg/mm²		Rmax n.d.
		kd 3,4E-3 s⁻¹		kd n.d.
		Kd 56,3 nM		Kd n.d.

Anti-Drug Antibody Quantification from Blood Serum

Anti-drug antibody (ADA) titers can be quantified by GCI directly from blood serum for immunogenicity detection.

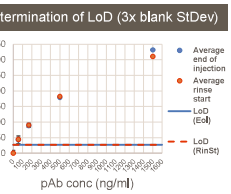
As shown here, in combination with robust microfluidics GCI allows for the detection of significantly lower antibody titers (lower limit of detection, LoD) by enabling direct quantification in almost pure blood serum.

Signals used for quantification



Concentrations spiked into mouse serum	
nM	ng/ml
10	1500
3.3	500
1.1	166.6
0.37	55.5

Determination of LoD (3x blank StDev)



Tested matrix	LoD in diluted samples	Effective LoD (in pure serum)
Buffer	25 ng/ml	Not applicable
10% blood serum	35 ng/ml	350 ng/ml
50% blood serum	40 ng/ml	80 ng/ml