Advancing Antibody Therapeutics: Leveraging Biolayer Interferometry for Label-Free Camelid Nanobody Quantitation and Kinetics Screening

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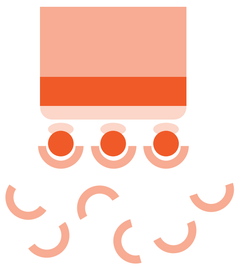
Introduction

In the antibody therapeutics landscape, immunization and antibody generation strategies have long been pivotal. Diversifying species for immunization- notably camelid nanobodies- offers broader and superior therapeutic potential.

Their minute size and robustness present excellent opportunities for companies to broaden the scope of targetable epitopes and biomanufacturing processes.

To support therapeutic applications, Gator® Bio has developed a universal nanobody biosensor capable of epitope binning and kinetic characterization on a BLI platform.

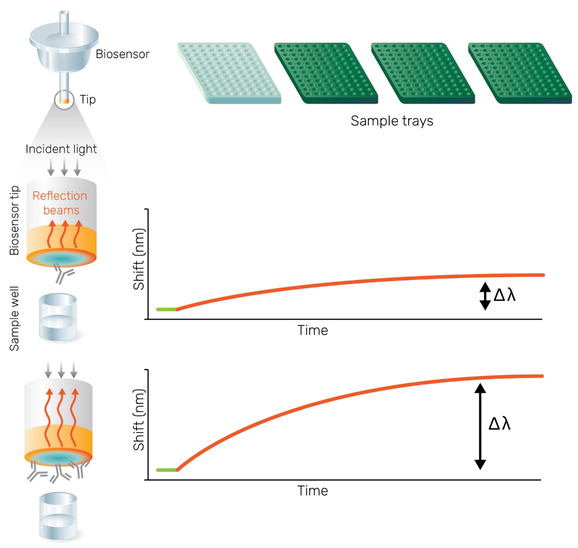
Introduction to Gator®



The Gator® instrument uses spectrometers to monitor real-time binding events to the biosensor. This enables label-free analysis of kinetics based on the biochemical composition of the base layer of the sensor.

Biolayer Interferometry Fundamentals

- Label-free technology based on reflection of light on the surface of a biosensor tip.
- The change in interference pattern is translated to nanometer shift and plotted against time.
- Shift is proportional to the number of biomolecules bound.



Gator® Next-Generation BLI

- Real-time analysis
- Minimal hands-on time
- Wide applications:
  - Protein-protein interactions
  - Therapeutics development
  - Viral vector analysis
- Tolerant to different buffers:
  - cell media, lysates, serum and plasma

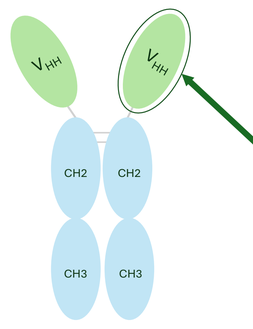
Instrumentation

All data collected for this study was generated using the Gator® Pro instrument. Capable of simultaneously performing 32 parallel binding reactions, it is the ideal choice for high throughput experiments, and can perform a 32x32 epitope binning study in under 8 hours.

Methods

Biosensor Design

Gator Bio's anti-VHH biosensor utilizes antibodies designed to bind conserved regions of VHH, enabling label-free binding of nanobodies for further characterization: quantitation, kinetics, and epitope binning– even in crude systems.



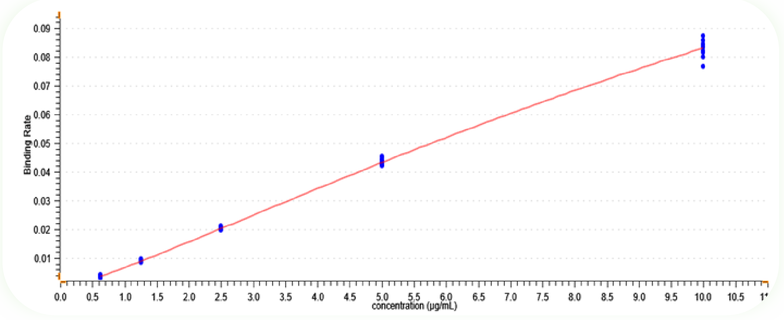
Camelid Origin

hcIgG
75 kDa
Heavy Chain IgG

VHH (Nanobody)
Variable domain of Heavy chain of Heavy-chain antibody)
<15 kDa

H-chain antibody variable region (Antigen binding region)

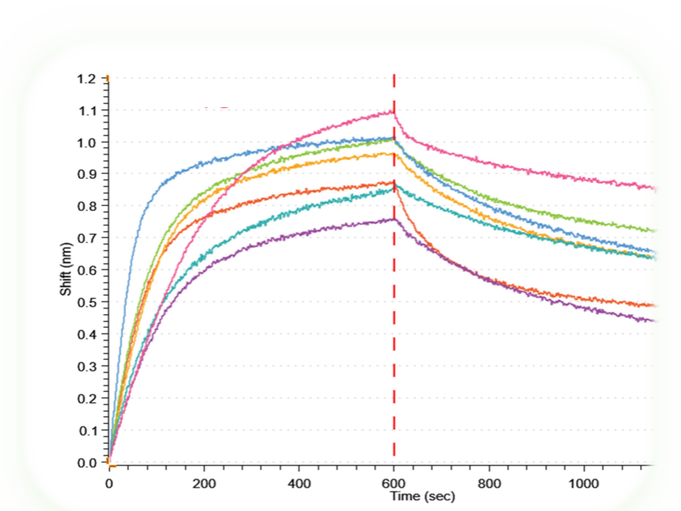
Nanobody Quantitation



Nanobody Back-Calculation (n=12)			
Known Conc. (µg/mL)	**Calculated Conc.** (µg/mL)	**Std Dev**	**CV (%)**
10	10.00	0.43	4.28
5	5.00	0.12	2.33
2.5	2.50	0.05	2.02
1.25	1.25	0.05	3.83
0.625	0.63	0.05	7.35

A stock solution of nanobody at a known concentration was diluted in Q Buffer to create a standard curve and measured values were back-calculated from a linear fitting.

Camelid Species Binding



Different camelid species loaded at 1 µg/mL show different immobilization affinities. Samples shown above: llama VHH and alpaca VHH

Nanobody Screening Results

Screening Method

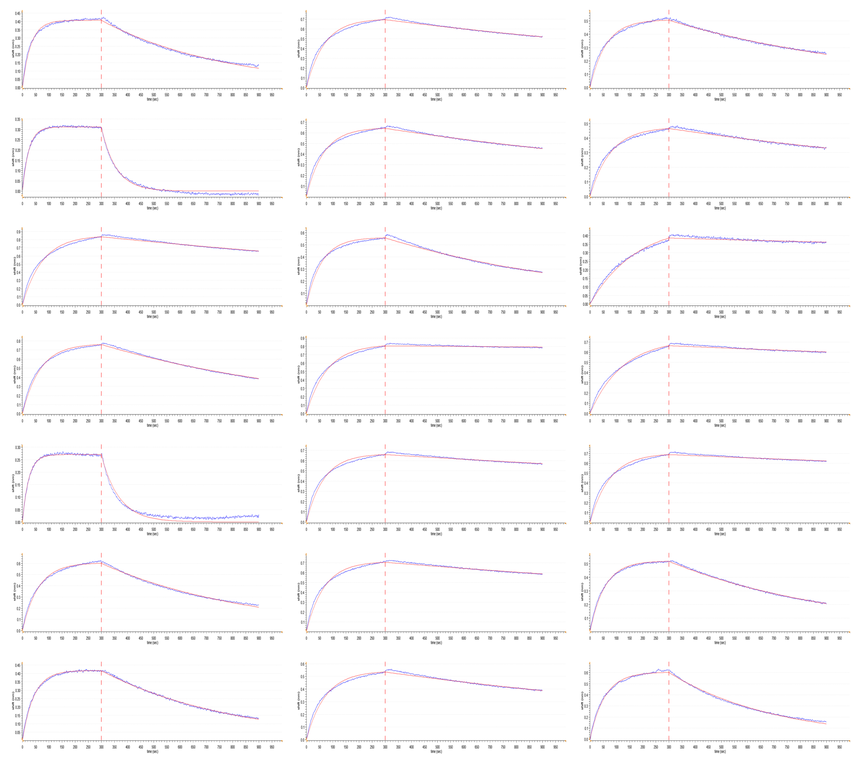
Nanobodies generated against the same target were loaded on anti-VHH biosensors, followed by a wash step to stabilize the surface.

Target antigen was loaded at 10 µg/mL concentration.

All steps were performed using Gator Bio's Q Buffer as diluent and running buffer.

Off-rate Analysis

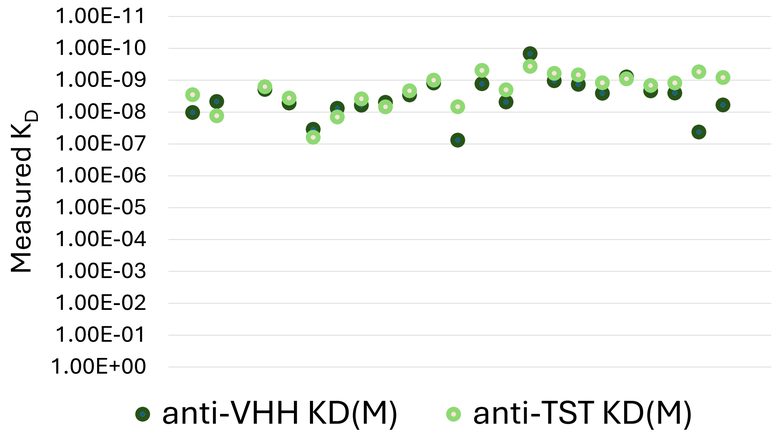
Collected data was fit to a 1:1 Langmuir kinetics model in Gator® analysis software to elucidate k_{off}.



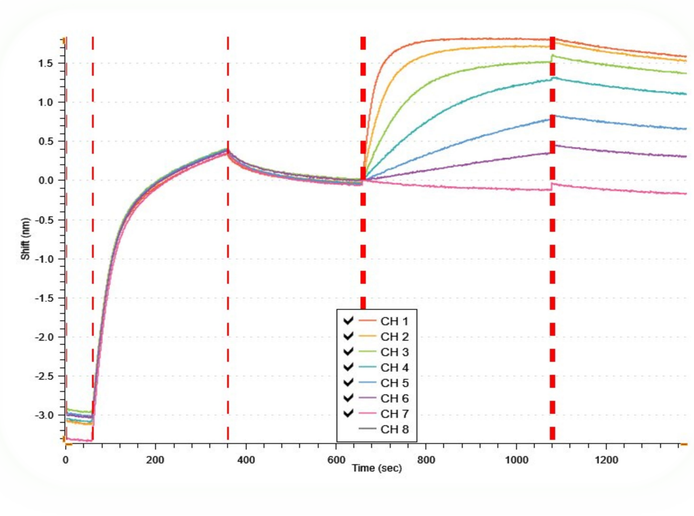
Label-Free vs Tagged Immobilization

The kinetics screening experiment was repeated using Gator'sepitope® StreptactinXT biosensor and the obtained K_D values were compared across both immobilization strategies.

K_D Comparison with Anti-StrepTag Biosensors



Characterization in Crude Lysate



User-generated data depicts capture of nanobody from *E. coli* lysate and subsequent binding kinetics. With Gator's® biosensors, a 15 kDa nanobody generated 2.5 nm loading signal height.

Conclusion

- Quantitation experiments demonstrates high accuracy across evaluated concentration range.
- Rapid kinetic screening of camelid antibodies raised against the same target.
- K_D values correlate to alternative capture strategies.
- Capable of isolating and characterizing directly from crude *e. coli* lysate.