High-Throughput (HTP) label-free Kinetics Screening using Gator-Pro in Early single domain antibody Discovery: A Comparative Analysis with Phage Display Samples from Periplasmic extract

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Introduction

- The Gator® Pro System is a high-throughput BLI platform featuring 32 high-frequency channels. This enables simultaneous kinetic screening and ranking of up to 1,300 monoclonal antibodies or 96 clones in parallel.
- It delivers accurate kinetic data without the need for multiplexing spectrometers, ensuring reliable results with each run while significantly reducing experimental complexity.
- Designed for ease-of-use and efficiency, the system requires less than 80 µL of each sample. Its simple layout formats remove the need for complex plate maps or multiple reagent aliquots, streamlining sample preparation.
- With full automation and minimal hands-on time, along with powerful data analysis software that rapidly fits and validates large datasets, Gator® Pro streamlines the workflow for antibody discovery and interaction studies.
- Akeagen, Inc. developed CaMouse™, a genetically modified mouse strain producing single domain antibodies (sdAbs), designed to replace camelids in sdAb discovery.
- This study explores the use of The Gator® Pro System , label-free Bio-Layer Interferometry (BLI) methodology for rapid, high-throughput kinetics screening of phage display-derived antibodies from CaMouse™ periplasm, enabling fast and detailed analysis of binding kinetics and epitope interactions.
- By comparing BLI-derived kinetic data with traditional screening methods, the study demonstrated BLI's sensitivity, reliability, and scalability, showcasing its potential to streamline and enhance early-stage antibody discovery workflows.

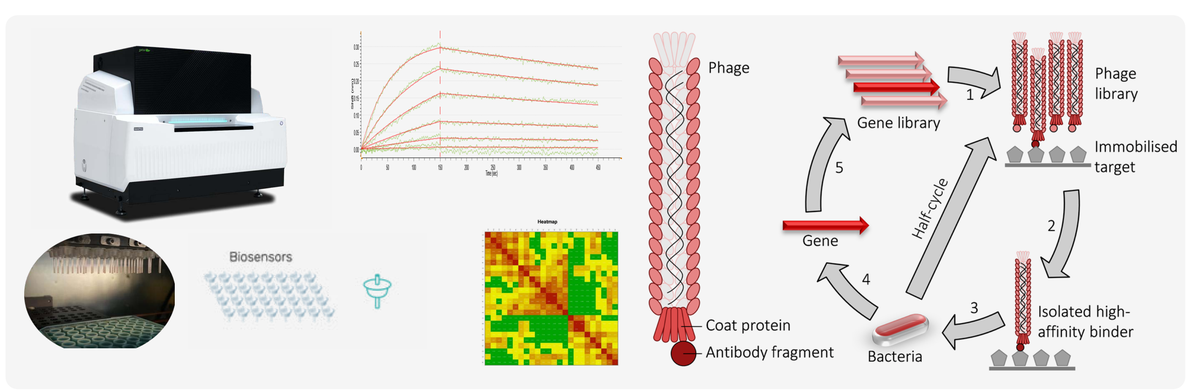

Figure 1: Gator® Pro is a 32-multiplexing BLI system that has been utilized to screen immune VH Phage Library and panning

Akeagen, Inc. is a private biotech company specializing in single domain antibody (sdAb) discovery solutions for life science research and next-generation biotherapeutics development. Its proprietary CaMouse™ in vivo rodent sdAb platform is distinguished by several advanced features. These include the largest number of endogenous VH genes (144) paired with an engineered endogenous IgG constant locus, enabling an exceptionally high immune response capability. The CaMouse™ platform also produces 100% heavy chain-only IgG (HcAb) in vivo and offers the highest diversity of in vivo single domain antibody repertoires, making it a powerful tool for antibody discovery and development. The novel single domain antibody discovery, facilitates sdAb application in life science research and next generation biotherapeutic development, such as bipspecifics, CAR-T, ADC, and Radiotherapeutics.

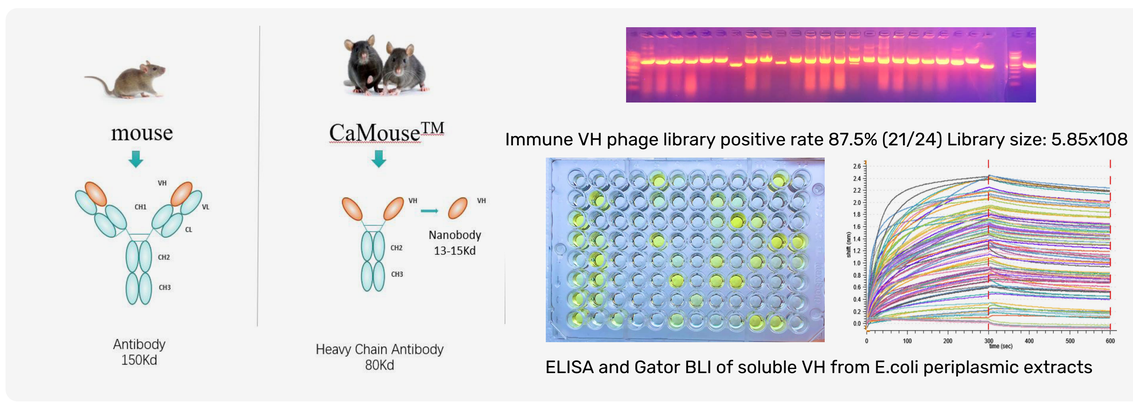

Figure 2: Akeagen, Inc provides single domain antibody (sdAb) discovery solution for next generation biotherapeutics development using its proprietary CaMouseTM in vivo rodent sdAb platforms.

Biolayer Interferometry (BLI) Solutions from Gator

- Label-free technology based on reflection of light on the surface of a biosensor tip
- The shift in interference pattern plotted against time when a molecule is bound
- The change in pattern is proportional to the number of biomolecules bound
- Gator® next-generation BLI is a versatile real-time analysis platform
- Minimal hands-on time
- Wide applications ranging from protein-protein interactions, therapeutics development and viral vector analysis
- Tolerant to different buffers, cell media, crude lysates, serum and plasma
- Ready to use Biosensors for versatile application

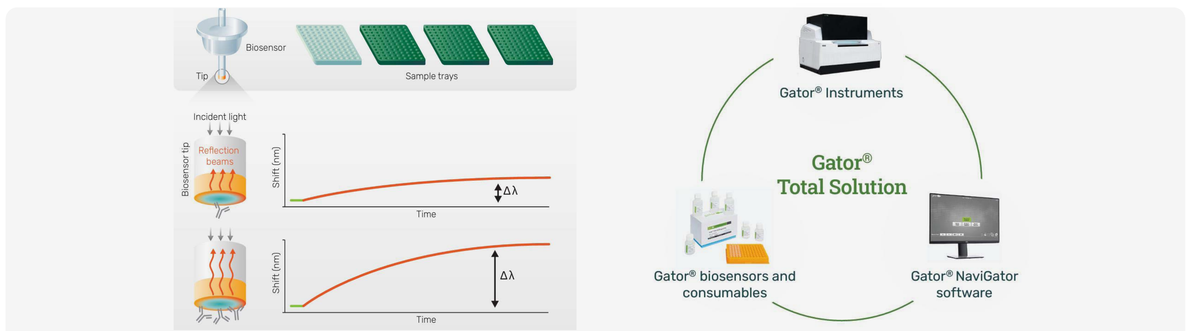

Figure 3: Gator® next-generation BLI, a versatile real-time analysis platform solution for label-free analysis

Gator® Probes	Function	Quantitation	Q Dynamic Range	Kinetics	Epitope Binning	Regeneration	
AAVX and AAV9	Binds serotypes AAV1-AAV8 and AAV10	+++	10^9 - 10^{13} vp/mL	+	-	++	No Salt
AAV High Sensitivity Kit (AAVX/AAV9)	High sensitivity quantitation of AAV serotypes	+++	$5x10^6$ – $5x10^{10}$ vp/mL	-	-	-	-
AAVX Empty/Full Kit	Enables % full determination	+++	0%-100% Full	-	-	-	-
Protein A	Binds human, mouse, and rabbit IgGs	+++	0.02 - 2000 µg/mL	+	+++	+++	Salt
Protein G	Binds human, mouse and rabbit IgGs	+++	0.02 - 2000 µg/mL	+	+++	+++	Salt
Protein L	Binds strongly to immunoproteins via kappa light chains	+++	0.1 - 2000 µg/mL	+	+++	+++	Salt
Anti-Human IgG Fc	Human IgG or Fc tag binding	+++	0.05 - 300 µg/mL	++	++	++	Salt
Anti-Human IgG Fc Gen II	Human IgG or Fc-tag binding	+++	0.3 - 6000 µg/mL	+++	+++	+++	No Salt
Anti-Mouse IgG Fc	Human IgG or Fc-tag binding	+++	0.02 - 2000 µg/mL	+++	+++	+++	No Salt
Anti–Human FAB	Binds F(ab), F(ab')2, Fc receptor, and full-length Human IgG	+++	0.3 - 3000 µg/mL	+++	+++	++	No Salt
IgM Probes	Immobilizes Rat, Mouse, and Human IgMs	+++	0.1-100 µg/mL in buffer 0.4-300 µg/mL in media	++	+++	++	No Salt
Anti-VHH	Binds to conserved region of VHH nanobodies of any species	++	Varies by species	++	+	++	No Salt
Anti–His	Binds His-tagged proteins	+	Protein-dependent	+	++	+	Salt
Ni-NTA	Tris-chelated Ni^{2+} ions binding to His-tagged proteins	+	Protein-dependent (Typically 0.25 – 1000 µg/mL)	++	++	++	Kit
Strep-Tactin XT	Binds twin-strep-tagged proteins	++	Protein-dependent	+++	+++	+++	No Salt
Streptavidin (SA)	Binds biotinylated targets	+	-	++++	-	-	-
SMAP	Streptavidin probe for small molecule and peptide detection	+	-	++++	-	-	-
SA XT	Streptavidin Probe for liposomes and protein complexes	+	-	++++	-	-	-
Flex SA	Binds biotinylated targets Reactivate the sensor surface!	++	-	++++	++	+++	Kit
Aminopropylsilane (APS)	Binds hydrophobic proteins	+	-	++	-	-	-
Amine-Reactive	Covalently attach amine group of proteins using EDC/NHS	+	-	++	++	-	-
Custom	Custom made biosensors (Anti-Rabbit, Anti-Rat, Anti-FLAG, Anti-GST, Anti-PEG)	Varies	Varies	Varies	Varies	Varies	

Table 1 : Summary of biosensor solutions from Gator®Bio

Materials & Method

Figure 4: Direct capture of the antigen via its Twin-Strep-tag enables efficient screening of periplasmic samples. This specific interaction with Strep-Tactin XT minimizes false positives. Following a similar assay format, SA XT biosensors were used in multiple experiments to determine off-rate kinetics and assess receptor blocking.

- Instrument used: Gator Pro
- Sensors used: Strep-Tactin XT Probes, PN#160003 & SA XT probes, Gator Bio, PN#160029
- Assay diluent: K buffer, PN# 120011

Assay set-up parameters & steps:

No	Step	Reagent	Time (sec)	RPM
1	Pre-wet	K buffer	300	Shaker A:0; Shaker B: 1000
2	Baseline	K buffer	120	1000
3	Loading	Antigen	200	1000
4	Baseline	K buffer	60	1000
5	Baseline	K buffer	60	1000
6	Association	Periplasmic sample	180	1000
7	Dissociation	K buffer	1000	1000

Table 2: Summary of Assay Steps

Schematic of a kinetics assay

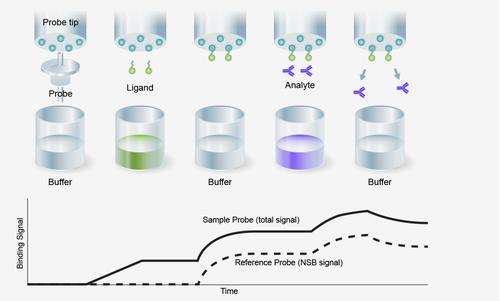

Figure 5: Schematic of a kinetic assay set-up using Gator BLI

Result Summary

Key Activities in Lead Identification:

1. Off-rate ranking:
- Measure how tightly and specifically the antibody binds to its target antigen in this case we tested three different types of antigens (A, B, or A/B) against 1300+ periplasmic samples.
- High-affinity and selective antibodies are typically prioritized as leads.

2. Receptor Blocking:
- The Samples binding to antigen A/A & A/B were tested against the receptor , in this HTP assay format the antigen was loaded followed by the receptor binding and it followed the periplasmic solutions. The ones that did not show any binding were receptor blockers & the once's that showed binding were non-blockers. This information gave diversity to the periplasmic samples.

3. Epitope binning:
- Similarly, the samples testing positive for binding to antigens were tested against a biosimilar in the same format as the receptor blocking experiment to further extract for information regarding each periplasmic sample. In the Figure 5, the flow chart summarizes the data flow.

4. Comparing the results with FRET & ELISA:
- In this study we compared the hit selection to the ELISA screening data where each plate was evaluated with the off-rate ranking data. Another orthogonal technique FRET assay was also evaluated against the Gator generated date.

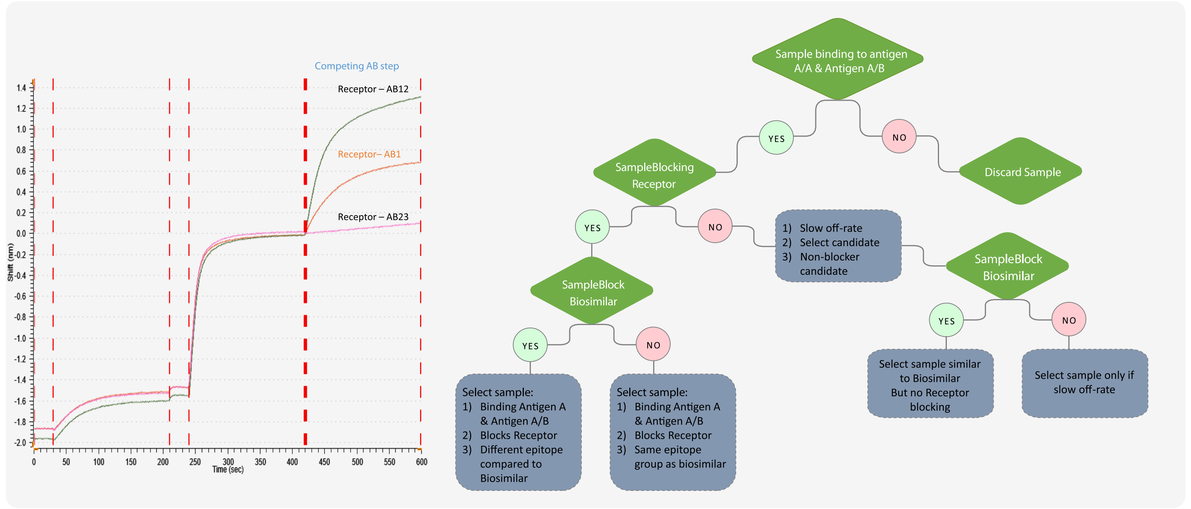

Figure 6: The left panel shows the actual assay steps during a receptor blocking assay. The right panel presents a flowchart summarizing the periplasmic sample screening strategy, where assays including antigen binding, receptor blocking, and biosimilar epitope binning were used to characterize over 1300 periplasmic extracts, enabling selection based on cumulative property information.

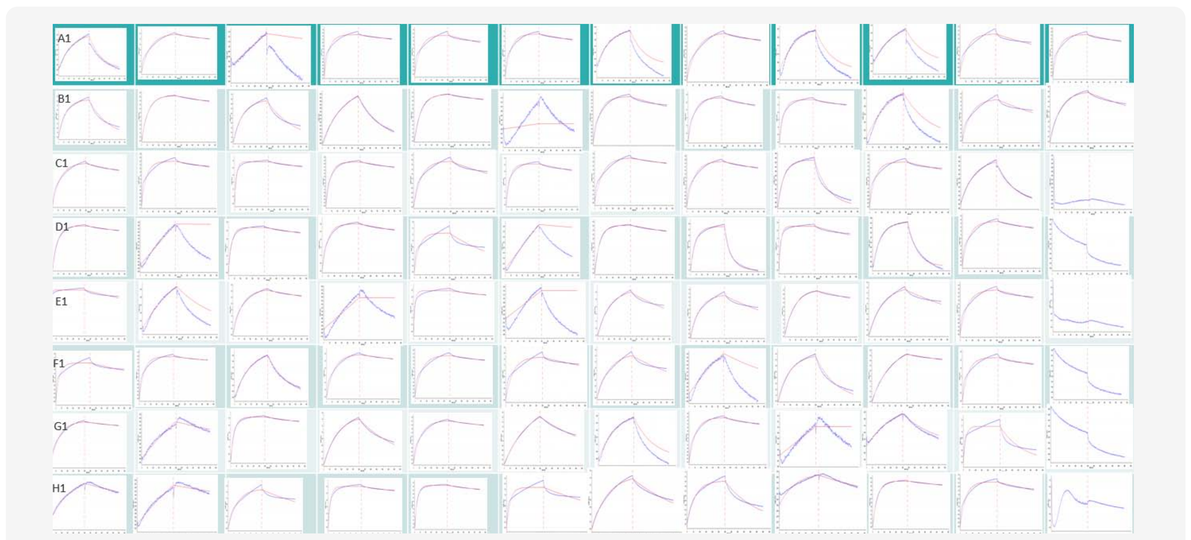

Figure 7: Summary of the screening results for all 96 samples from a single plate. The software efficiently fits the kinetic data for 96 or 384 samples simultaneously, requiring minimal processing time.

Conclusion

Key Activities in Lead Identification:

- High-Throughput BLI Platform: GatorBio's Bio-Layer Interferometry (BLI) platform provides advanced, large-scale protein characterization capabilities, accelerating efficient lead selection.
- Comprehensive Binding Analysis: A versatile suite of biosensors enables detailed evaluation of general binding properties and precise kinetic interactions between drug candidates and their targets.
- Rapid Data Processing: The platform features intuitive software designed for rapid, high-throughput data analysis, significantly reducing time-to-results.
- Integrated Multi-Parameter Assays: BLI assay design supports the simultaneous assessment of multiple parameters—such as off-rate ranking, epitope binning, receptor blocking, and cross-reactivity—within a single assay format.

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