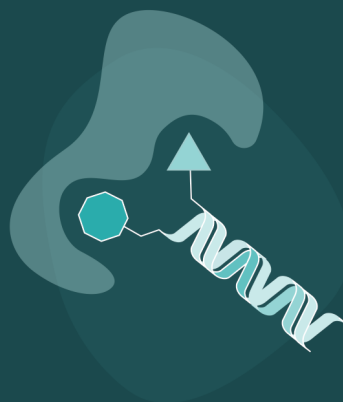
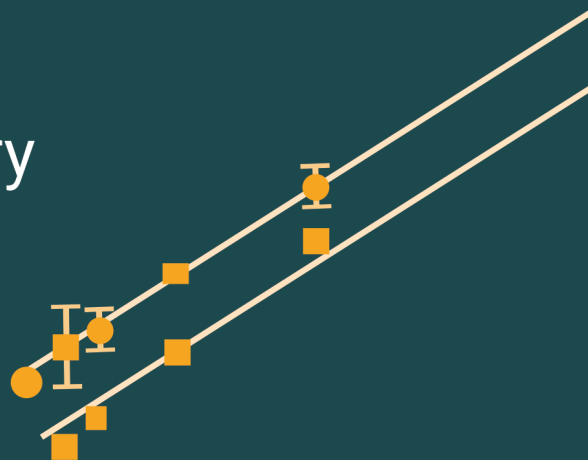
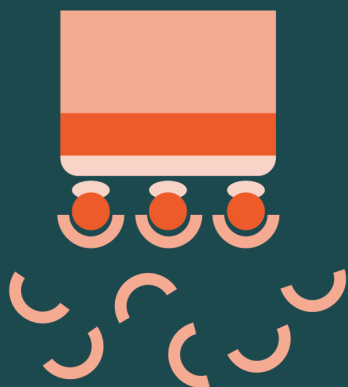




WHITE PAPER

# Next-Gen BLI Technology

Accelerating Antibody Discovery



gator 

## Accelerating Antibody Discovery through Gator® BLI Biosensor Technology

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### Authors

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### Introduction

Developing and engineering antibodies for diagnostics or therapeutics necessitates comprehensive characterization of an antigen-antibody interaction.

Biolayer Interferometry (BLI) technology has greatly helped speed up the process of antibody discovery. Gator Bio's BLI platforms and its biosensors offer ease of use, reliability, and high precision analysis when compared with commonly used immunoassays, such as an enzyme-linked immunosorbent assay (ELISA).

Information on binding kinetics, concentration, competitive binding to a specific epitope, and affinity is key for the identification of potential targets from tag-free molecules or molecules with widely used tags.

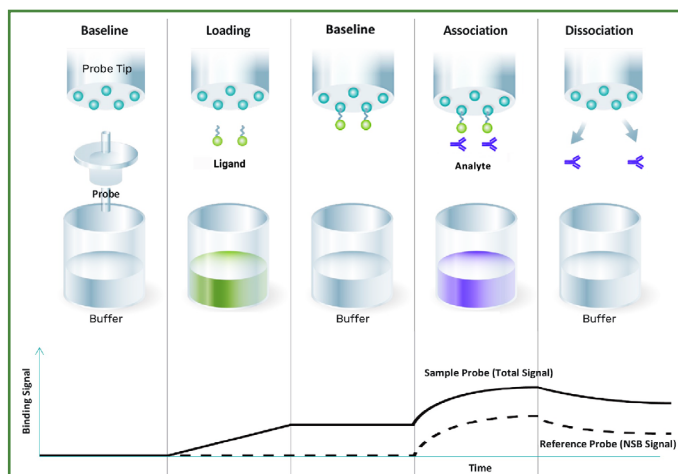
Here, we present data using selected five biosensors, Anti-Human IgG Fc Gen II (HFCII), Anti-Mouse IgG Fc (MFC), Ni-NTA, Streptavidin (SA), Flex Streptavidin (Flex SA), and Small Molecule Analysis Probes (SMAP), from Gator Bio's portfolio of biosensors that can significantly increase the throughput for protein/small molecule quantification and characterization of antigen-antibody interactions, thereby advancing the selection of lead diagnostic and therapeutic antibodies.

### Biosensor Features

- Precoated and ready-to-use
- Enhanced sensitivity and reduced nonspecific binding
- Useful for quantitation, kinetics, and epitope binning with BLI technology
- Suitable for a vast array of sample type, including crude (cell lysate and media) and purified proteins
- Stable over a broad pH range
- Can be regenerated or reactivated
- Samples can be reused among different biosensors

# Accelerating Antibody Discovery through Gator® BLI Biosensor Technology

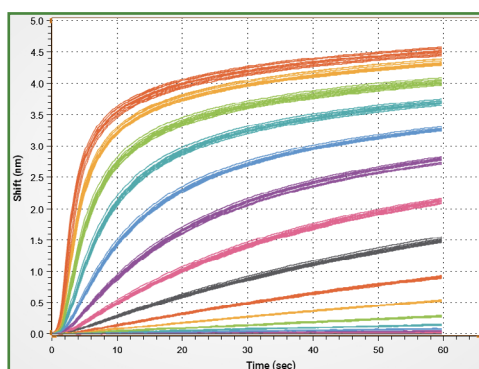
## Schematic of a Kinetics Assay



**Figure 1.** Overview of a kinetics assay workflow and sensogram with BLI technology. Real-time measurements are recorded as the change in the wavelength of reflected light returning from the optical biosensor surface. First, Gator® precoated biosensors are equilibrated by dipping into wells containing the assay buffer. In the ligand loading step, biosensors are dipped into wells containing the antigen. After a wash, the ligand-loaded biosensors are placed into wells containing the analyte, and an association or binding signal is measured. After another dip into wells containing the assay buffer, the ligand dissociates from the precoated biosensors. From the GatorOne analysis software, the association constant ( $k_{on}$ ), the dissociation constant ( $k_{off}$ ), and the  $K_D$  and affinity values are obtained.

## Anti-Human IgG Fc GEN II BIOSENSOR

1. High performance HFCII biosensor that detects all four isotypes of human IgG.
2. Enhanced dynamic range for quantification, more stable baseline for kinetics, and better regeneration capabilities.
3. Significantly improves productivity, throughput, and accuracy of quantitation and real-time kinetics, resulting in faster lead selection from antibody screening.



**Figure 2.** Quantitation of human IgG with concentrations ranging from 0.3 - 6000 µg/mL using Gator® HFCII biosensors.

# Accelerating Antibody Discovery through Gator® BLI Biosensor Technology

Performance Summary	
Dynamic Range	0.3–6000 µg/mL in Q Buffer; 1–2000 µg/mL in diluted cell culture media
Limit of Detection	0.1 µg/mL
Time to Results	8 samples/2 min; 96 samples/34 min
Cost-Effective	Reusable for at least 20 regenerations
Sample	Crude tolerant

Table 1. Performance summary of Gator® HFCII biosensors in Quantitation (Q) and Kinetics (K) Buffer, and media)

Regeneration Cycles	k <sub>off</sub> (1/s)	k <sub>on</sub> (1/Ms)	K <sub>D</sub> (M)
1	2.82E-03	3.70E+05	7.62E+09
5	2.66E-03	4.08E+05	6.53E-09
9	3.19E-03	4.39E+05	7.27E-09
13	3.73E-03	4.46E+05	8.37E-09
17	4.46E-03	4.46E+05	9.99E-09
20	3.91E-03	4.97E+05	7.86E-09

Table 2. Regeneration performance of Gator® HFCII biosensors. Kinetics parameters for anti-receptor binding domain (RBD) IgG1 and RBD protein for over 20 regeneration cycles. k<sub>off</sub>, k<sub>on</sub>, and K<sub>D</sub> values are within 20X folds of each other.

## Anti-MOUSE IgG Fc BIOSENSOR

1. Gator Bio's MFC biosensor detects antibody concentrations (0.02 – 2000 µg/mL), providing an expanded dynamic range for rapid antibody screening without dilution steps. Exceeding what traditional methods can produce, next-gen biosensor shows no compromise in data quality with even greater sensitivity, providing faster results and significant cost savings in the antibody sector.
2. Gator® next-gen biosensor maintains high sensitivity and reproducibility even after 10 regenerations, making it very cost-effective.

	Gator® BLI Platform	Other BLI Platform
Dynamic Range	0.02 – 2000 µg/mL	1 – 100 µg/mL
Throughput	8 samples/30 sec	8 samples/120 sec
Regeneration	20X	Not capable
Limit of Quantitation	0.02 µg/mL	1 µg/mL

Table 3. Performance at a glance.

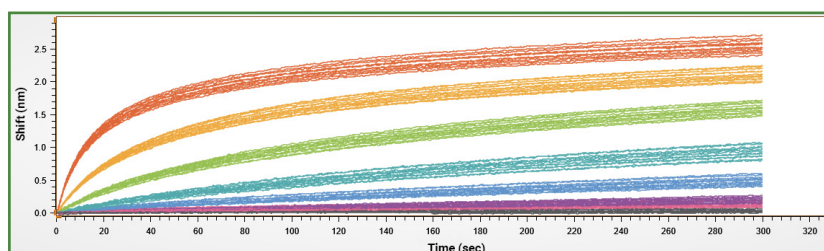


Figure 3. Reproducibility after 10 regenerations using MFC biosensors and a serial dilution of mouse IgG standards from 0.01 - 10 µg/mL.

# Accelerating Antibody Discovery through Gator® BLI Biosensor Technology

## Ni-NTA BIOSENSOR

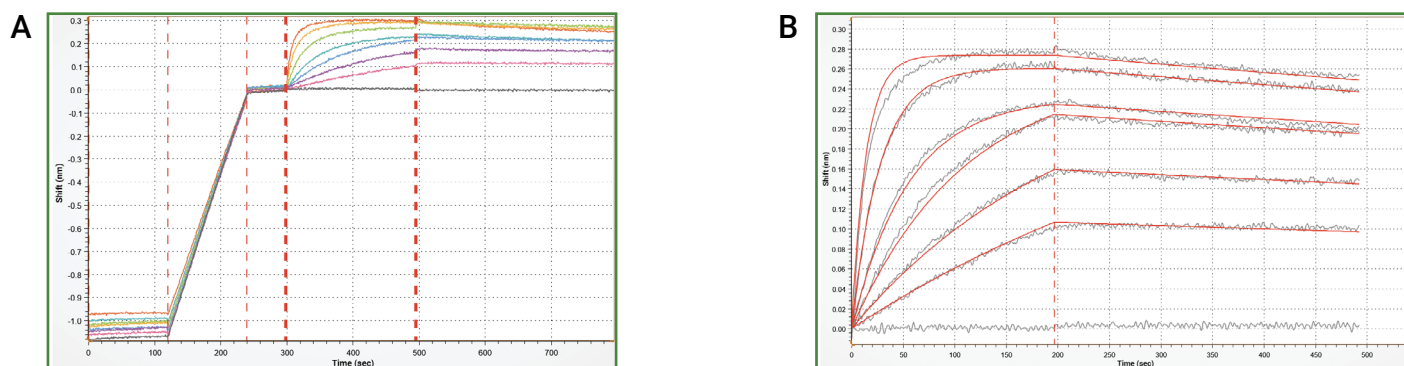
1. Functionalized with Qiagen™ Tris-NTA and charged with Ni<sup>2+</sup> ions for high affinity immobilization of His-tagged proteins; no Ni<sup>2+</sup> recharging step needed with the use of Ni-NTA Regen and Neutral Buffers.
2. Stable immobilization of His-tagged proteins allows for throughput kinetics and epitope binning of antibodies, enabling rapid and continuous quantification without the need for Ni<sup>2+</sup> recharging.

	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6	Ab7	Ab8	Ab9	Ab10	Ab11	Ab12
Ab1	-0.015	0.446	0.082	0.29	0.395	0.465	0.126	0.434	0.078	0.329	0.423	0.538
Ab2	0.59	0.112	0.697	0.821	0.855	0.086	0.775	0.118	0.661	0.793	0.826	0.084
Ab3	0.275	0.667	0.143	0.715	0.875	0.704	0.305	0.666	0.152	0.711	0.841	0.701
Ab4	0.057	0.255	0.116	0.155	0.237	0.303	0.087	0.255	0.113	0.196	0.249	0.331
Ab5	0.023	0.117	0.075	0.08	0.114	0.153	0.051	0.129	0.082	0.12	0.139	0.21
Ab6	0.551	0.206	0.713	0.868	0.899	0.153	0.797	0.201	0.697	0.815	0.843	0.11
Ab7	0.153	0.555	0.093	0.336	0.423	0.571	0.161	0.53	0.096	0.343	0.408	0.564
Ab8	0.843	0.13	0.715	0.836	0.858	0.087	0.787	0.118	0.691	0.799	0.805	0.08
Ab9	0.356	0.802	0.176	0.785	0.915	0.791	0.325	0.737	0.159	0.718	0.828	0.723
Ab10	0.092	0.3	0.107	0.172	0.239	0.353	0.094	0.288	0.115	0.191	0.239	0.35
Ab11	0.041	0.152	0.071	0.107	0.135	0.198	0.061	0.164	0.096	0.13	0.136	0.217
Ab12	0.801	0.214	0.714	0.811	0.826	0.126	0.761	0.199	0.699	0.779	0.785	0.123

**Figure 4.** 12x12 antibody epitope binning assay using Ni-NTA biosensors for over 12 regenerations without the need for Ni<sup>2+</sup> recharging. Red = Competition, Yellow = Ambiguous, and Green = Non-competition.

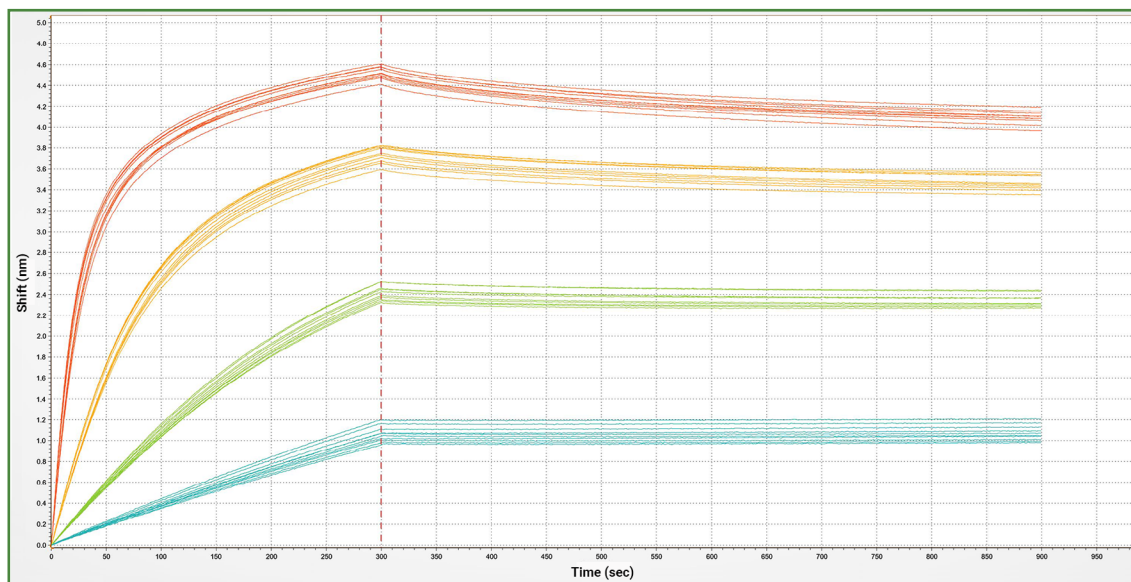
## STREPTAVIDIN (SA) AND FLEX SA BIOSENSORS

1. Captures biotinylated proteins (molar coupling ratio of <3) or proteins with AviTag™.
2. Gator® Flex SA Kit is the first in the market to provide reactivable streptavidin biosensors. The ability to reuse biosensors in different applications is desirable for a cost-effective research program. The same biosensor can be used to capture different biotinylated proteins after reactivation without loss of performance.



**Figure 5A-B.** (A) Affinity measurement for protein-protein interactions. After a baseline measurement in Gator® K Buffer, biotinylated rabbit IgG was loaded onto SA biosensors, followed by an association and dissociation of an antigen from 0 - 500 nM. (B) Global-fit analysis using GatorOne software for antibody-antigen interaction, as seen in A. K<sub>D</sub> = 2.27 nM (r<sup>2</sup> = 0.99).

## Accelerating Antibody Discovery through Gator® BLI Biosensor Technology



**Figure 5C.** Kinetics characterization of biotinylated tumor necrosis factor (TNF)-α and anti-TNF-α for over 10 reactivations using Flex SA biosensors.

Biotinylated PDL1 : Anti-PDL1		Biotinylated CRP : Anti-CRP	
Reactivation 1	2.00E-10	Reactivation 2	4.77E-10
Reactivation 3	1.08E-10	Reactivation 4	4.28E-10
Reactivation 5	1.17E-10	Reactivation 6	3.88E-10
Reactivation 7	1.06E-10	Reactivation 8	3.49E-10
Reactivation 9	0.40E-10	Reactivation 10	3.22E-10
Average	1.14E-10	Average	3.93E-10

**Table 4.** KD measurements using the same set of Flex SA biosensors with two different kinetics pairs. The pairs were used alternately for over 10 reactivations.

### SMAP BIOSENSORS

1. Captures small molecules (down to 150 Da), peptides, and biomolecules. Provides high sensitivity and enhanced signal when needed.
2. Traditional BLI biosensors struggled with small molecule kinetics. Gator® SMAP biosensor exhibits significantly improved performance with greater loading and global fit.
3. Gator® sensograms exhibit clear binding signal and dissociation curves used to calculate  $k_{on}$ ,  $k_{off}$ , and  $K_D$  while traditional BLI displays no clear signal.



## Accelerating Antibody Discovery through Gator® BLI Biosensor Technology

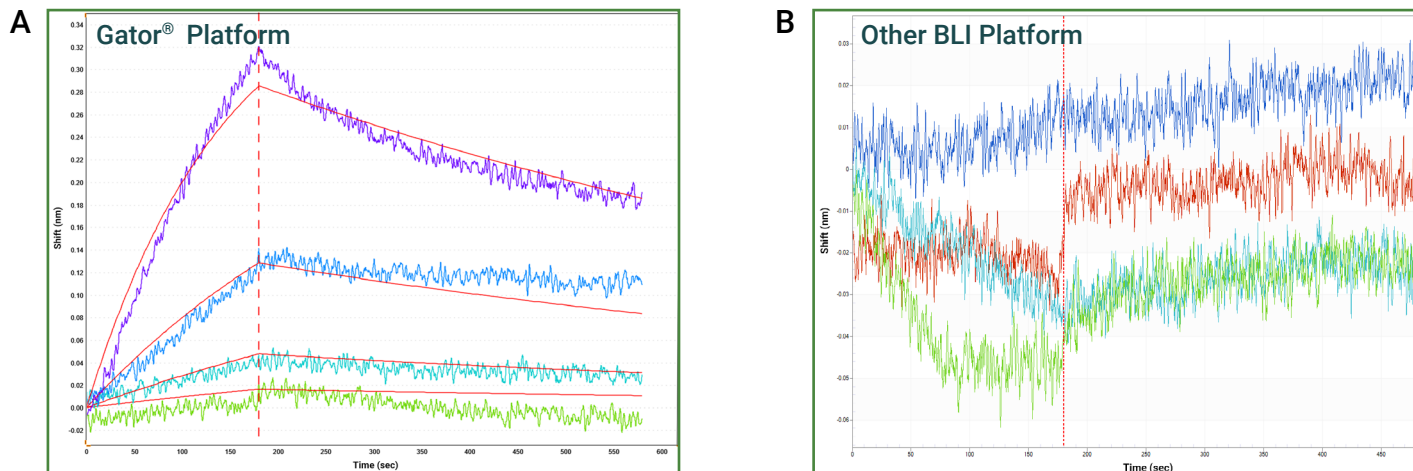


Figure 6A-B. Sensogram plots in a kinetics study comparing (A) SMAP biosensors on Gator Bio BLI platform versus (B) another BLI platform and their biosensors.

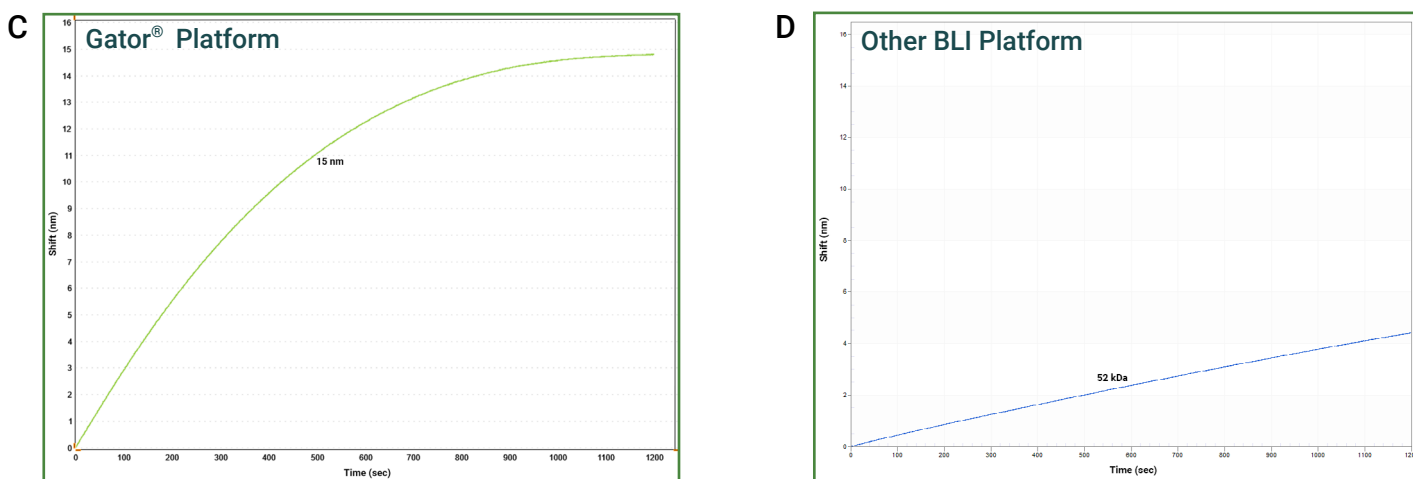


Figure 6C-D. Loading capacity of (C) SMAP biosensors on Gator Bio BLI platform versus (D) another BLI platform and their biosensors.

# Accelerating Antibody Discovery through Gator® BLI Biosensor Technology

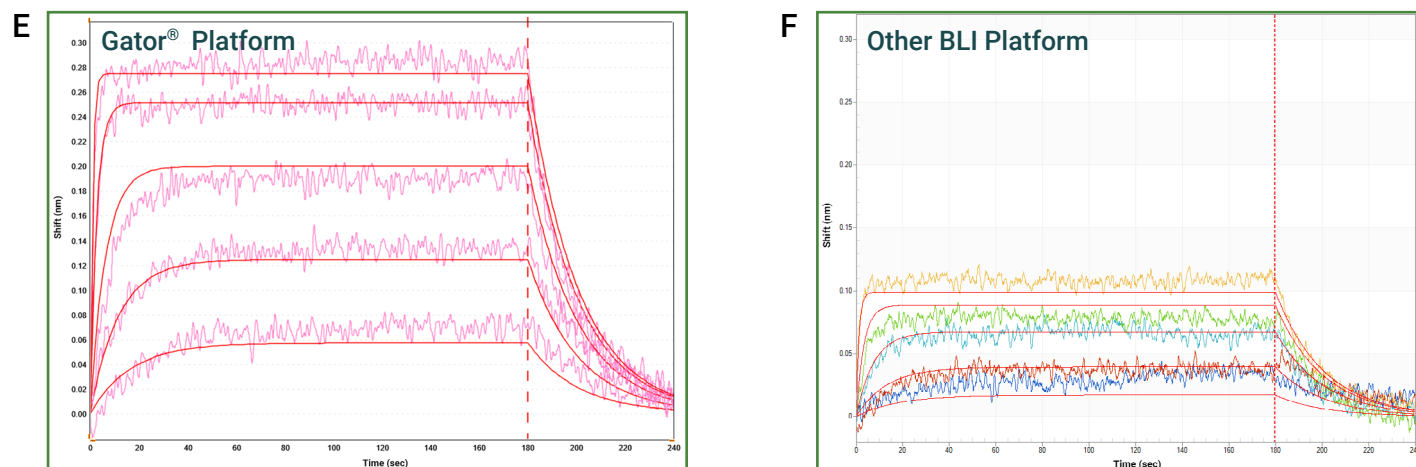


Figure 6E-F. Global-fit analyses comparing (E) SMAP biosensors on Gator Bio BLI platform versus (F) another BLI platform and their biosensors.

## SUMMARY

- Next-gen Gator® BLI platform provides a total solution for antibody discovery.
- Gator® instruments and biosensors work together to deliver precise, reliable, and reproducible data compared to other competitive BLI biosensors and systems.
- Binding kinetics, epitope binning, and affinity applications using Gator® biosensors are able to assess antibody-antigen interactions very efficiently.
- Gator® biosensors enable faster and more cost-effective discovery of antibodies for the diagnostic and therapeutic market.

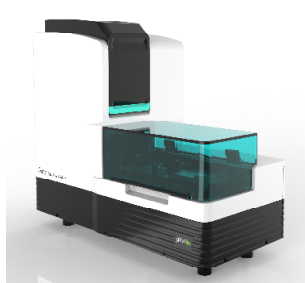
## GATOR BIO TOTAL SOLUTION

### Gator® Biosensors



+

### Gator® Prime



+

### GatorOne Software

