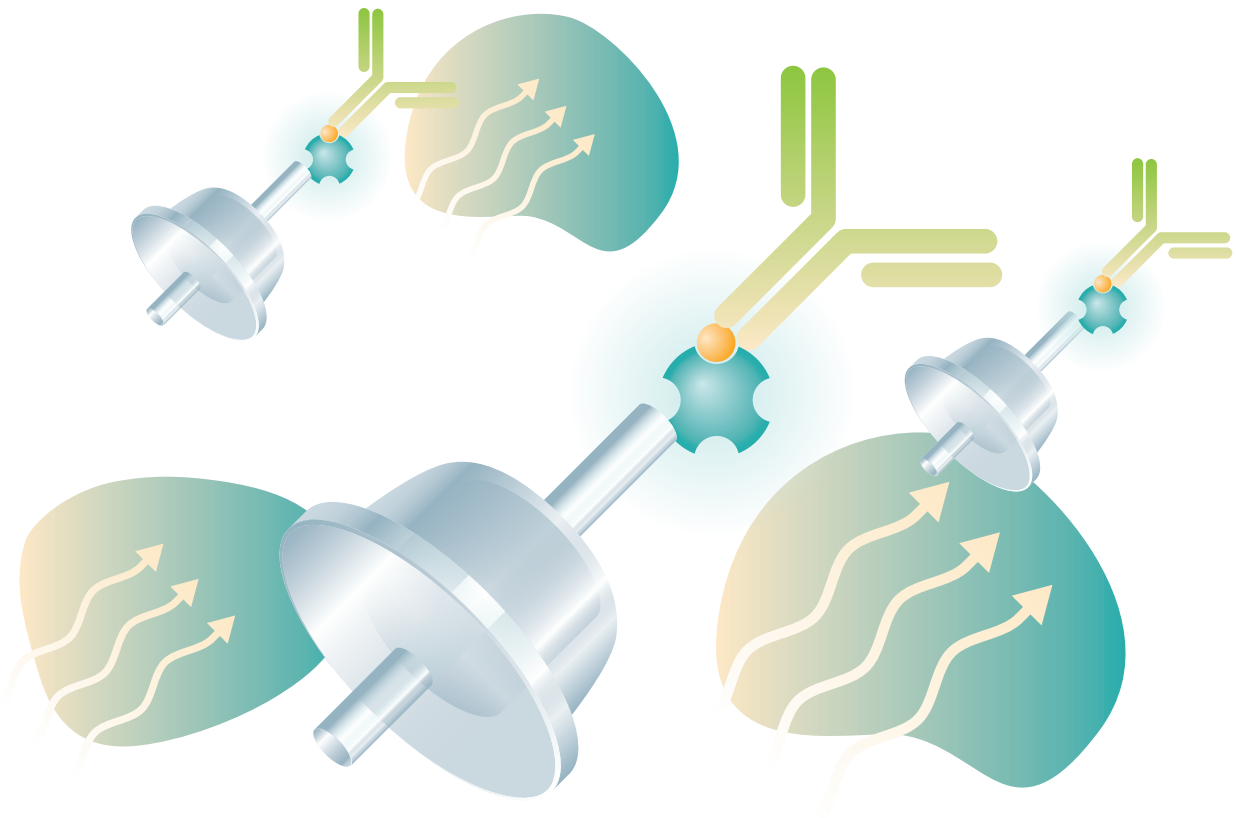
White Paper



Next-gen BLI streptavidin probes – Enabling kinetics from small molecules to LNPs

Purpose

This white paper provides an overview of the applications of Gator Bio's Streptavidin based probes and compares them to the other probes on the market.

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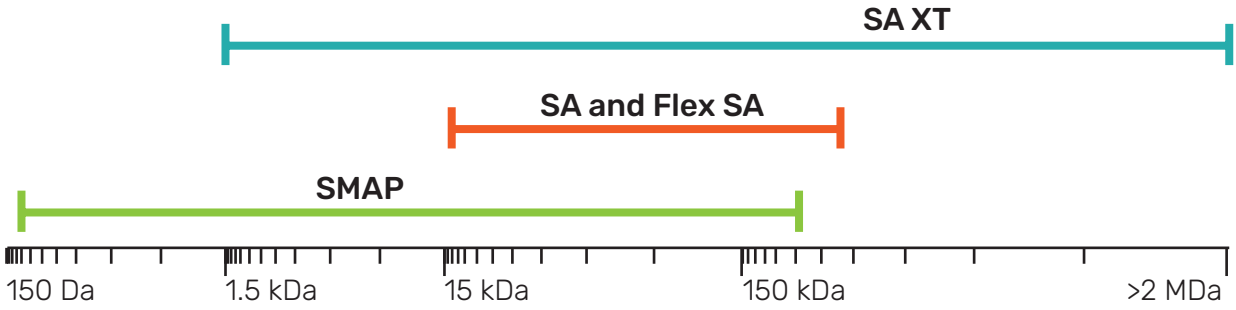
Introduction to Biolayer Interferometry

Biolayer interferometry (BLI) is a biophysical technique that measures the binding interactions between two molecules. The streptavidin biotin interaction is widely used in BLI, as it is highly specific and provides a strong non-covalent interaction. Streptavidin is a tetrameric protein derived from Streptomyces avidinii that binds biotin with extremely high affinity (dissociation constant $K_D = 10^{-15}$ M). Biotinylated proteins or target molecules are captured by streptavidin-coated probes, allowing for their detection and analysis. This provides a highly sensitive and specific method for characterizing protein-protein, protein-ligand, or antibody-antigen interactions.

Gator Streptavidin Probes

Gator Bio offers four different streptavidin (SA) sensors applicable to different biomolecules ranging from small molecules and peptides to large proteins and lipid nanoparticles. In addition to single use probes, Gator Bio also offers a reactivable SA biosensor that can be reused up to 40 times depending upon the protein. This helps to significantly lower the cost of analysis per sample without compromising data quality.



Streptavidin Probe	Part Number	Capture Molecule
SA	160002	Proteins 15-150 kDa
SMAP	160011	Small Molecules > 150 Da
SA XT	160029	High Sensitivity, Peptides, Proteins, Oligos, LNPs 1.5 kDa -2 MDa
Flex SA	350001	Reusable, Proteins 15-150 kDa

SA Probe

Gator Bio's SA probe is widely used for capture of biotinylated proteins in the molecular weight range 15-150 kDa. The proprietary chemistry used to coat the SA Probe provides reliable protein kinetics data.

Figure 1 shows a kinetics experiment between biotinylated programmed death ligand-1 (PD-L1) loaded at 1.5 µg/mL and anti PD-L1 at 0, 10, 30 and 100 nM. The k_{on} (1.5×10^5 M^{-1} s^{-1}), k_{off} (3×10^{-5} s^{-1}) and K_D (2×10^{-10} M) values are automatically calculated from the sensorgrams using the GatorOne software. Additionally, the low %CV (3.4%) observed from the multiple loadings of PD-L1 onto the probe surface indicates that sensor response is highly reproducible.

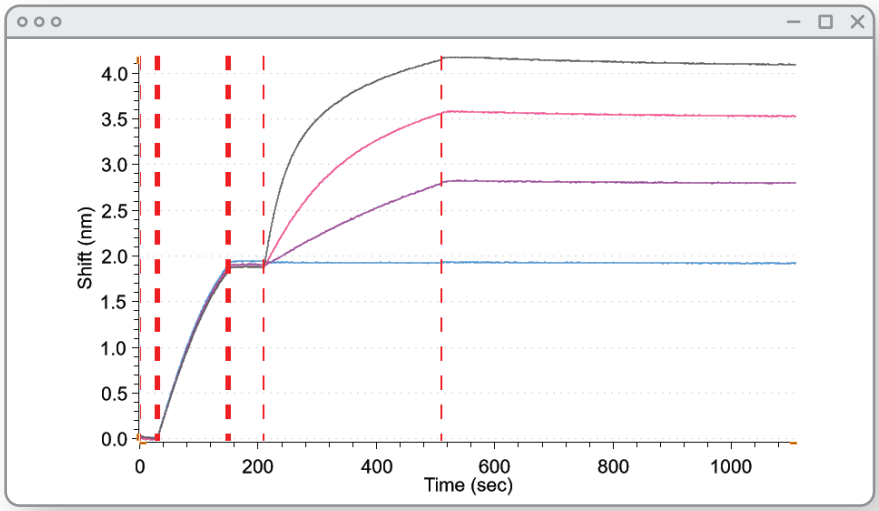


Figure 1: Kinetics using SA probe. *Sensogram showing kinetics between biotinylated PD-L1 at 1.5µg/mL loaded onto the SA probe and anti PD-L1 at 0, 10, 30, 100 nM. The %CV for loading of PD-L1 onto the four probes is 3.4 %. The k_{on}, k_{off} and K_D values are 1.5 x 10^5 M^{-1} s^{-1}, 3 x 10^{-5} $s^{-1}$and 2 x 10^{-10} M, respectively.*

Small Molecule Analysis Probe (SMAP)

Gator Bio's streptavidin-based Small Molecule Analysis Probe (SMAP) is designed for peptide and small molecule binding kinetics with molecules as small as 150 Da. The unique surface chemistry of the probes allows for high-capacity immobilization of biotinylated or Avi-tagged recombinant proteins on the surface. It generates higher signal for small molecule analytes binding to the captured protein as compared to competitor probes.

As shown in Figure 2, when the SMAP probe is used to measure the kinetics between biotinylated carbonic anhydrase II (loaded onto the probe) and small molecule furosemide (MW 330 Da), higher signal and better resolution are observed versus measurements using a competitor BLI from another vendor. Additionally, the SMAP probe provides kinetics measurements that are in excellent agreement with studies performed using surface plasmon resonance (Biacore system).

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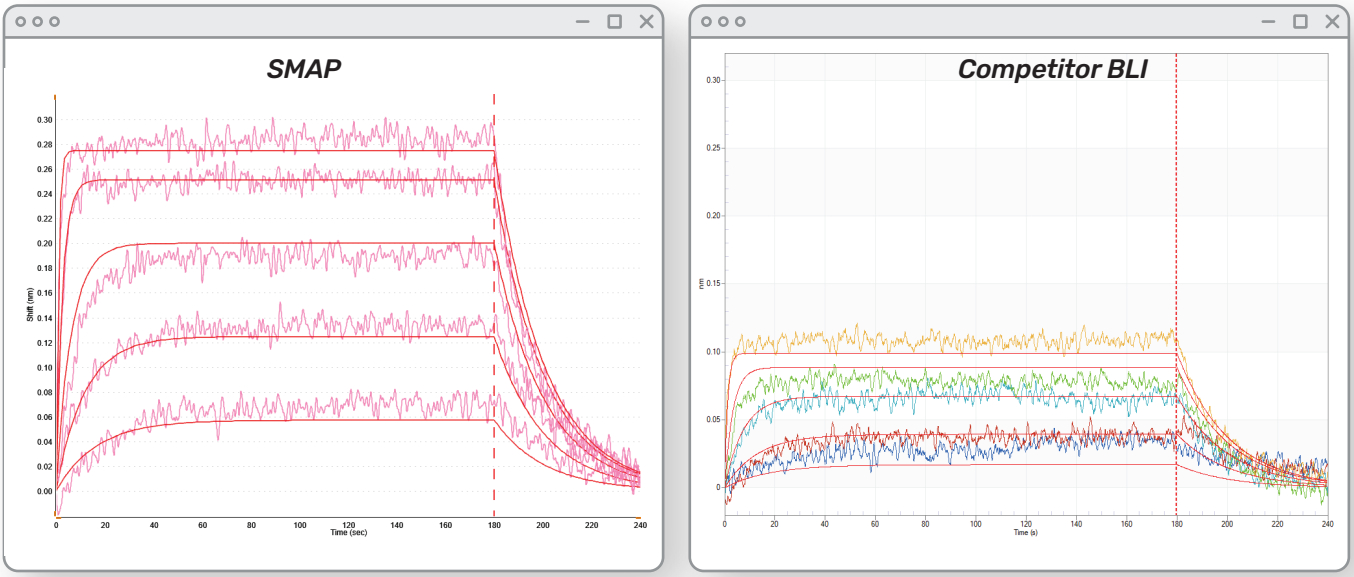

B

	SMAP probe	Biacore
k_{off} (1/s)	4.86E-02	4.96E-02
k_{on} (1/Ms)	1.00E+05	9.66E+04
K_D (M)	4.85E-07	5.13E-07

Figure 2. Kinetics comparison for furosemide (330 Da). *Comparison of different concentrations of furosemide binding with carbonic anhydrase II loaded onto the SMAP probe versus a competitor BLI system (A). SMAP probes give higher signal and better resolution between different concentrations. SMAP probes provide very similar kinetics data to Biacore studies (B).*

Another example, (Figure 3), shows the binding kinetics for fluorescein (MW 332 Da) with anti-fluorescein antibody. The SMAP probe (left) provides strong and measurable binding signals and dissociation curves for calculating k_{on}, k_{off}, and K_D, whereas kinetics measurements are difficult if not impossible using the competitor probe (right).

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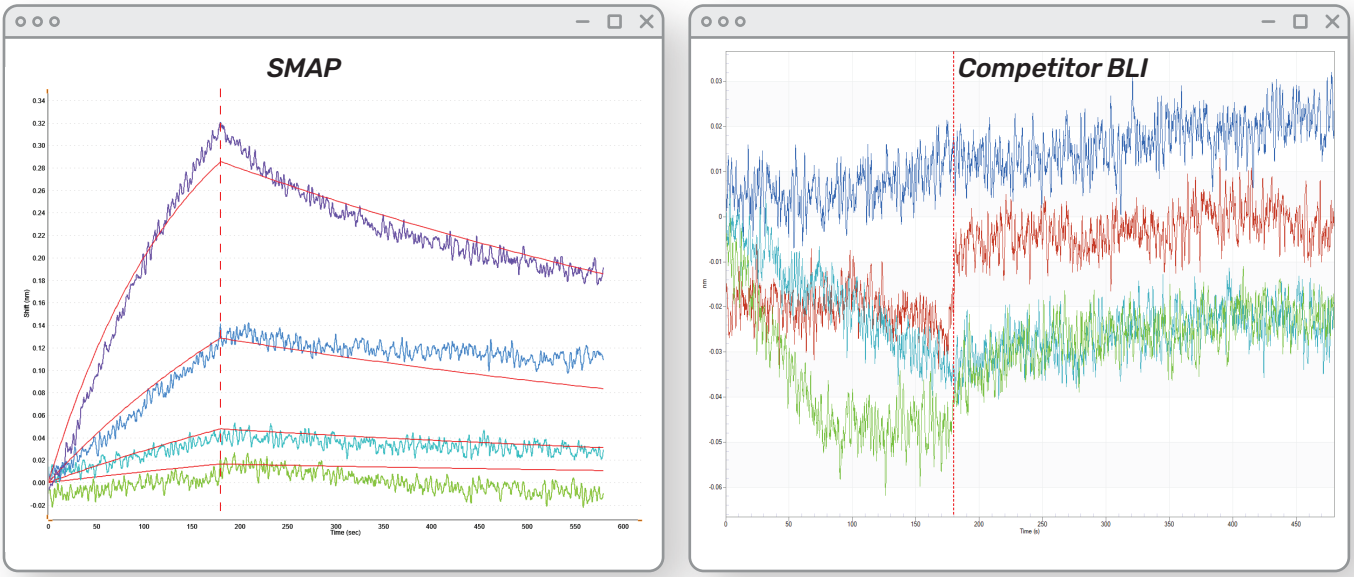
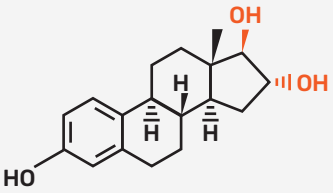
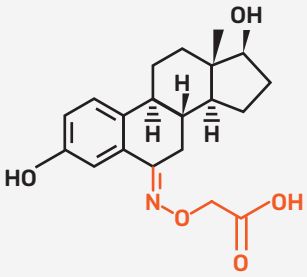


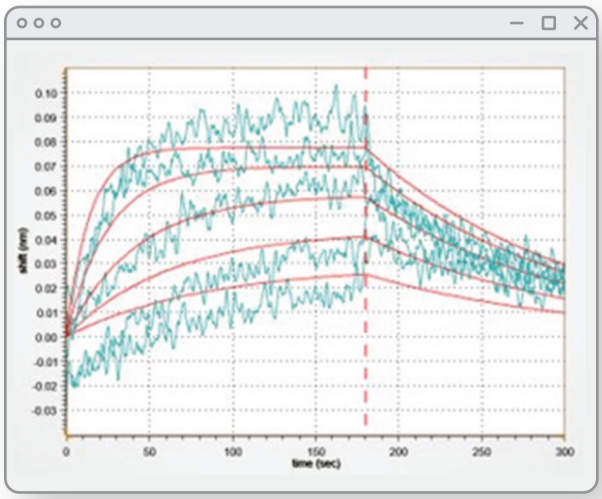
B

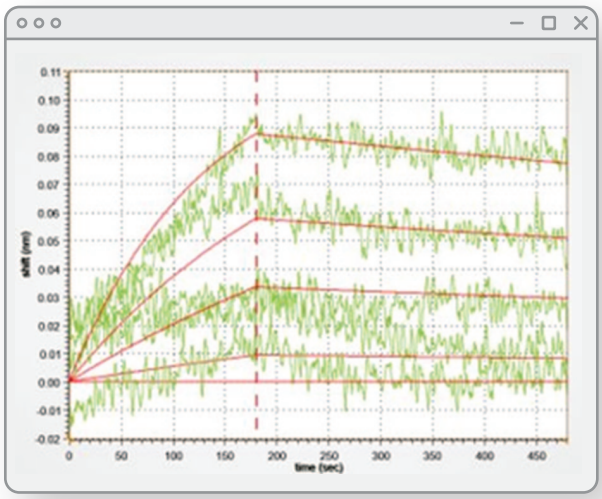
k_{off} (1/s)	1.54E-03
k_{on} (1/Ms)	5.79E+05
K_D (M)	2.66E-09

Figure 3. Binding comparison for fluorescein (332 Da). *Comparison of different concentrations of fluorescein binding with anti-fluorescein antibody loaded onto the SMAP probe versus a competitor BLI system (A). SMAP probes give higher signal and better resolution between different concentrations for accurate kinetic measurements (B).*

In the development of small molecule therapeutics, small structural changes can have a profound impact on the safety and efficacy of a drug. The SMAP probe is sensitive enough to detect small structural changes in small molecules that might affect binding affinity as is demonstrated by the binding of anti-estradiol antibody to estradiol and estradiol oxime, shown in Figure 4. Here small structural changes between these related hormones have a large impact on k_{on}, k_{off}, and K_D, equivalent to several orders of magnitude.

	Estriol (E3)	Estradiol oxime (E2 oxime)
		
k_{off} (1/s)	7.91E+05	1.08E+06
k_{on} (1/Ms)	8.83E-03	7.35E-05
K_D (M)	1.12E-08	6.81E-11





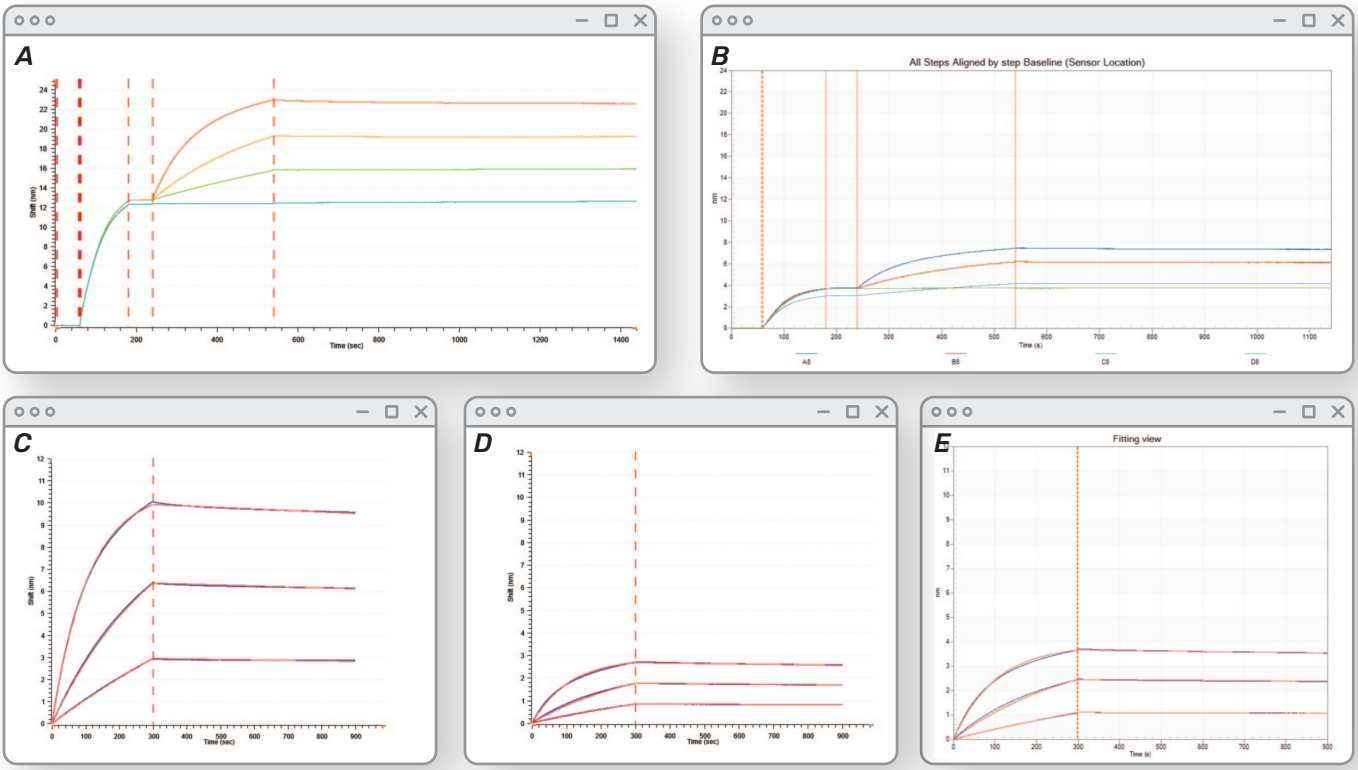
Estriol (E3) ***Estradiol oxime (E2 oxime)***

Figure 4. Binding of estrogen related molecules to anti-estradiol antibody. *The structures and the binding kinetics are shown for estradiol and estradiol oxime. Structural differences are highlighted in red. Representative sensorgrams for different concentrations of estriol and estradiol oxime are shown at the bottom. Small structural changes between molecules can have a large impact on the binding kinetics as shown by the different k_{on}, k_{off}, and K_D values.*

SA XT Probe

The recently launched SA XT probe provides enhanced signal strength and an extended molecular weight range for capture molecules. Due to a combination of unique optical properties of the probe material and a proprietary chemistry coated onto the probe, the SA XT sensor provides 3-4x higher signal than the SA probe and a competitor BLI probe from another vendor. This higher signal enables the use of lower concentrations of ligand and analyte, enabling measurements on highly diluted solutions and conserving precious samples for other studies.

Figure 5 compares the SA XT probe and a competitor BLI sensor from another vendor for measuring the binding of biotinylated mouse IgG and anti-IgG. The signal with the SA XT probe is 3-4x higher compared with the SA probe and competitor BLI sensor. As shown in the table, the loading signal percentage change from the competitor BLI sensor is more than 100% in all cases.

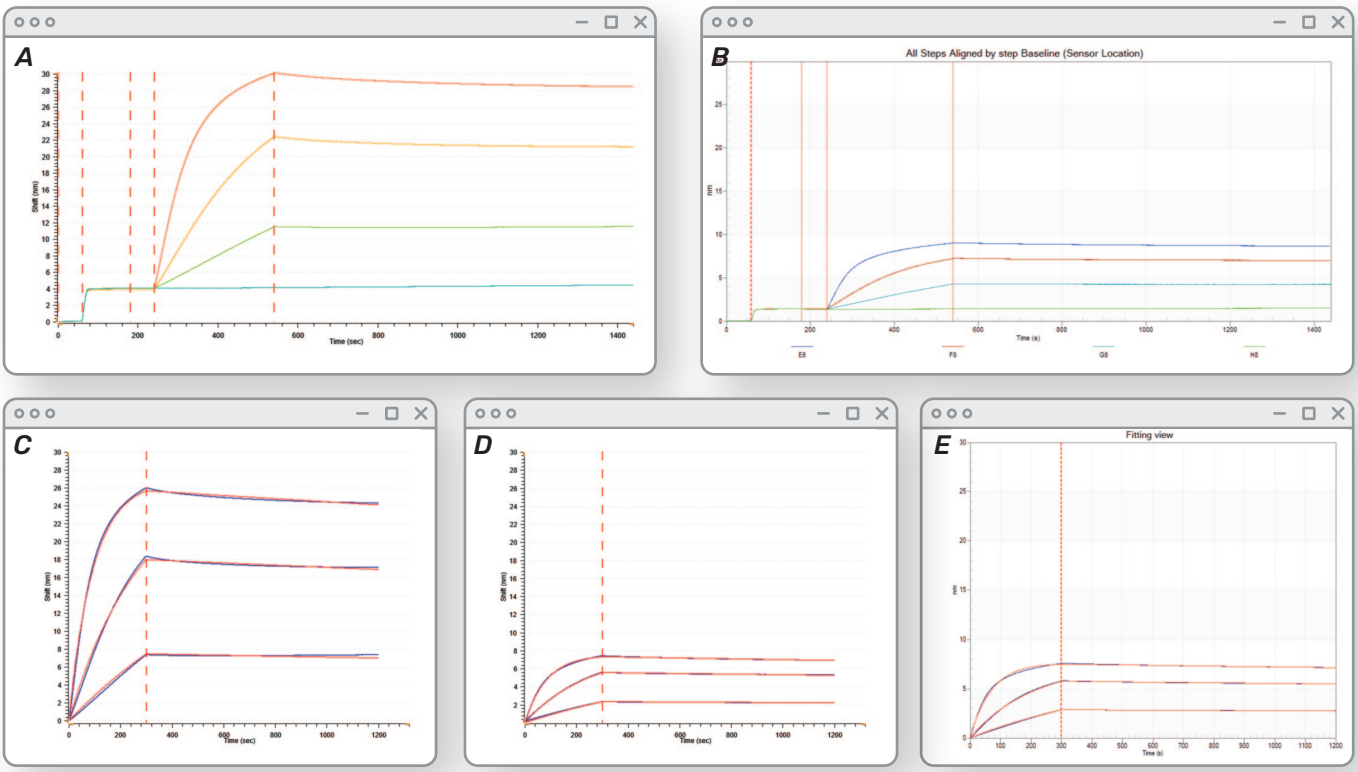


	mIgG (nm)	Anti-mIgG20 µg/mL (nm)	Anti-mIgG6.7 µg/mL (nm)	Anti-mIgG2.2 µg/mL (nm)	k_{off} (1/s)	k_{on} (1/Ms)	K_D (M)
Competitor BLI SA	3.42	3.63	2.40	1.05	5.9E-5	7.42E4	7.95E-10
SA	2.39 (-31%)	2.49 (-31%)	1.84 (-23%)	0.85 (-19%)	9.44E-5	6.03E4	1.6E-9
SA XT	12.58 (+268%)	10.06 (+177%)	6.38 (+166%)	2.93 (+179%)	5.03E-5	6.85E4	7.34E-10

Figure 5. Binding and kinetics comparison for biotinylated mouse IgG and anti IgG. *Top: Sensogram showing loading signals, association and dissociation using the SA XT probe (A) and a competitor BLI sensor from another vendor (B). Middle: Sensogram showing kinetics experiment using the SA XT probe (C), SA probe (D) and a competitor BLI sensor from another vendor (E). Bottom: Table comparing loading signals and binding kinetics using the competitor BLI SA sensor from another vendor, the SA probe, and the SA XT probe. Percentages represent the percent difference from the competitor BLI SA sensor.*

Similar to the binding of larger proteins, the SA XT sensor provides at least 3x higher loading signal with small biotinylated peptides and subsequent antibody binding compared to other BLI streptavidin sensors. This broadens its range of use, enabling reliable kinetic analysis and off rate screening of antibodies against biotinylated peptide ligands as small as 1.5 kDa.

Figure 6 shows the binding of biotinylated troponin 1 peptide fragment (TnI) (2.3 kDa) and anti-TnI. Strong, reproducible binding is observed for reliable calculation of k_{on}, k_{off}, and K_D values.



	Peptide loading (nm)	Anti-TnI 20 µg/mL (nm)	Anti-TnI 6.7 µg/mL (nm)	Anti-TnI 2.2 µg/mL (nm)	k_{off} (1/s)	k_{on} (1/Ms)	K_D (M)
Competitor BLI SA	1.35	7.54	5.72	2.81	5.47E-5	1.09E5	5.03E-10
SA	0.81 (-40%)	7.43 (-2%)	5.59 (-2.2%)	2.34 (-17%)	6.41E-5	8.18E4	7.27E-10
SA XT	4.1 (+204%)	21.3 (+182%)	13.96 (+144%)	6.45 (+130%)	8.47E-5	1.01E5	8.42E-10

Figure 6. Binding and kinetics comparison for biotinylated TnI (2.3 kDa) and anti TnI. *Top: Sensogram showing loading signal, association and dissociation using the SA XT probe (A) and a competitor BLI sensor from another vendor (B). Middle: Sensogram showing kinetics experiment using the SA XT probe (C), SA probe (D) and a competitor BLI sensor from another vendor (E). Bottom: Table comparing loading signals and binding kinetics using the competitor BLI SA sensor from another vendor, the SA probe, and the SA XT probe. Percentages represent the percent difference from the competitor BLI SA sensor.*

The SA XT probe is capable of measuring binding for a wide range of molecules. Figure 7 shows the loading signal for various compounds including oligonucleotides, peptides, proteins, and antibodies. In all cases, the loading signal vastly surpasses competitive BLI technologies enabling much more sensitive and accurate measurements and binding studies at very low concentrations.

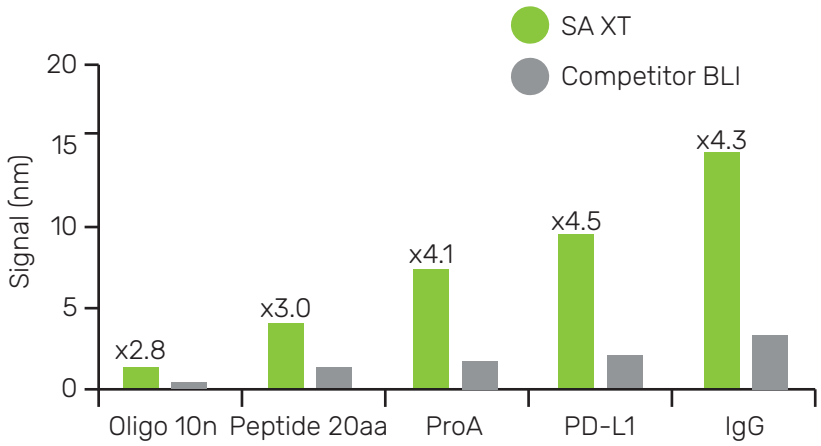


Figure 7. Loading signal comparison. *Comparison of loading signals between the SA XT probe and the competitor BLI sensor from another vendor. Loading signals are shown for a range of molecular weights including a biotinylated oligonucleotide (6.15 kDa), biotinylated TnI (20-40) (2.2 kDa), biotinylated ProA (70 kDa), biotinylated PD-L1 (72 kDa), and biotinylated mouse IgG (150 kDa).*

Due to its optical properties and unique surface chemistry combined with the improvements in the algorithm underlying Gator software, the SA XT sensor is valuable for the kinetic analysis of large macromolecules. With competitor BLI technology, the binding of macromolecules can result in troponin 1 peptide fragment. This is presumably caused by large changes in optical thickness of the biological layer that result in large shifts in the interference pattern. With the SA XT probe, a positive binding signal is maintained even with thicker biolayers up to 0.7 µm.

The capacity of the SA XT probe for studying large biomolecules is demonstrated in Figure 8 for lipid nanoparticles (LNPs) with molecular weights in the MDa range. Here the SA XT probe is loaded with a biotinylated antibody against a moiety on the surface of LNPs. Because of the high sensitivity of the SA XT probe, the initial loading of the LNP produces a stronger signal compared to a competitor BLI probe. Moreover, during the subsequent antibody association, the signal stays positive for the SA XT sensor but inverts for the competitor probe.

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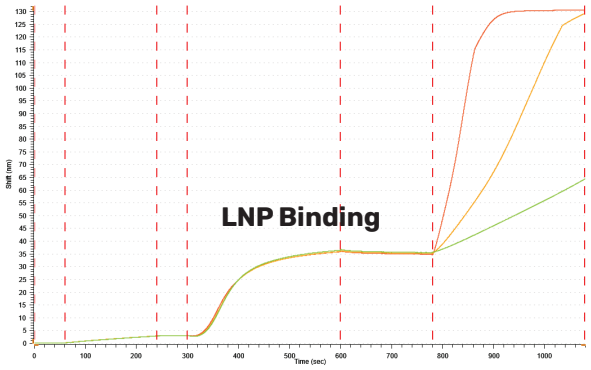

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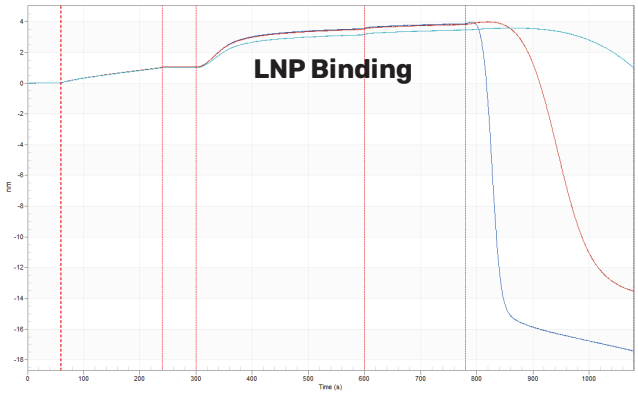


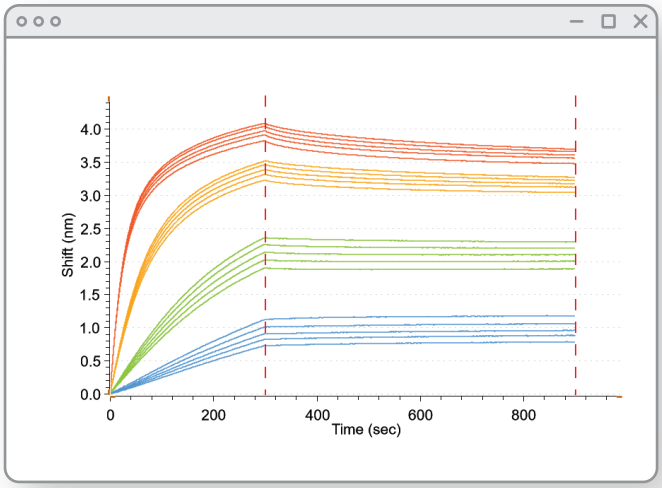
Figure 8. Lipid nanoparticle kinetics. *Graphs show a kinetic experiment between a lipid nanoparticle (LNP) with a diameter of 100 nm (MDa range) and an anti-LNP antibody. Following a baseline measurement in K Buffer, biotinylated antibody against a moiety on the LNP surface was loaded onto (A) SA XT and (B) competitor BLI SA probe. Following another baseline in K buffer, the LNP was loaded, which gives a higher signal on SA XT. Subsequent antibody binding shows a positive signal on SA XT and an inverted signal on the traditional BLI probe*

Flex SA – A reusable streptavidin probe

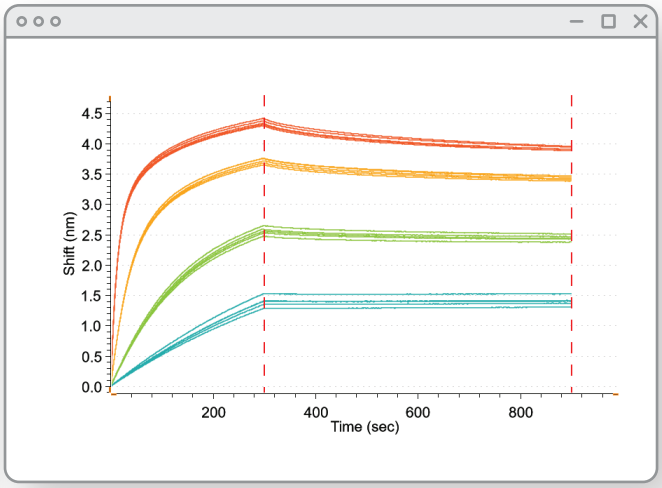
Conventional streptavidin sensors cannot be regenerated and reused because of the tight binding between biotin and streptavidin. Gator Bio has developed the Flex SA streptavidin biosensor that can be reactivated. With the Flex SA sensor, the streptavidin itself is stripped off the surface and fresh streptavidin is coated onto the surface, allowing the sensor to be reused multiple times. The sensor provides robust performance over at least 10 reactivations with CV <10%, and depending upon the protein, the sensor can be reactivated up to 40 times. The reagents needed for reactivation are provided as part of the Flex SA kit.

The binding response for biotinylated tumor necrosis factor -α (TNF-α) and anti-TNF-α is shown in Figure 9 over multiple reactivations. As shown, the signal is highly reproducible at each concentration from the first set of measurements (1-5) to the last set of measurements (26-30). Average loading height, standard deviation, and %CV for 4 different Flex SA probes over 30 reactivations shows excellent signal strength and reproducibility with %CV < 10%. This enables the calculation of accurate binding kinetics even over many repeated reactivations.

A: Reactivation 1-5



B: Reactivation 26-30



C: Loading Height

	Probe 1	Probe 2	Probe 3	Probe 4
Loading height Avg (nm)	0.704	0.694	0.672	0.683
Loading height SD	0.053	0.047	0.048	0.051
Loading height %CV	7.52	6.77	7.08	7.47

D: Kinetics Measurements

K_D **Avg**	7.6E-10 M
K_D **SD**	1.67E-10

Figure 9. Reactivation using the Flex SA probe. *Sensogram showing kinetics experiment for biotinylated TNF- α and anti- TNF-α using four different Flex SA probes. The blue, green, orange, and red curves represent 10, 30, 100, and 300 nM, anti-TNF-α respectively. Overlaid curves in the same color represent reactivations at different concentrations. The table (C) shows the loading height statistics over the 30 reactivations for each probe. Additionally, the average KD and standard deviation over 30 reactivations is shown (D).*

Because the Flex SA probe reactivates completely, it is not necessary to designate a separate probe for different proteins. Thus, the Flex SA probe can be used to load a different protein after each reactivation making it a highly versatile tool. This is possible because of the complete removal of streptavidin from the probe surface during reactivation. Loading fresh streptavidin creates a clean and uncontaminated surface on the probe that can bind any biotinylated protein.

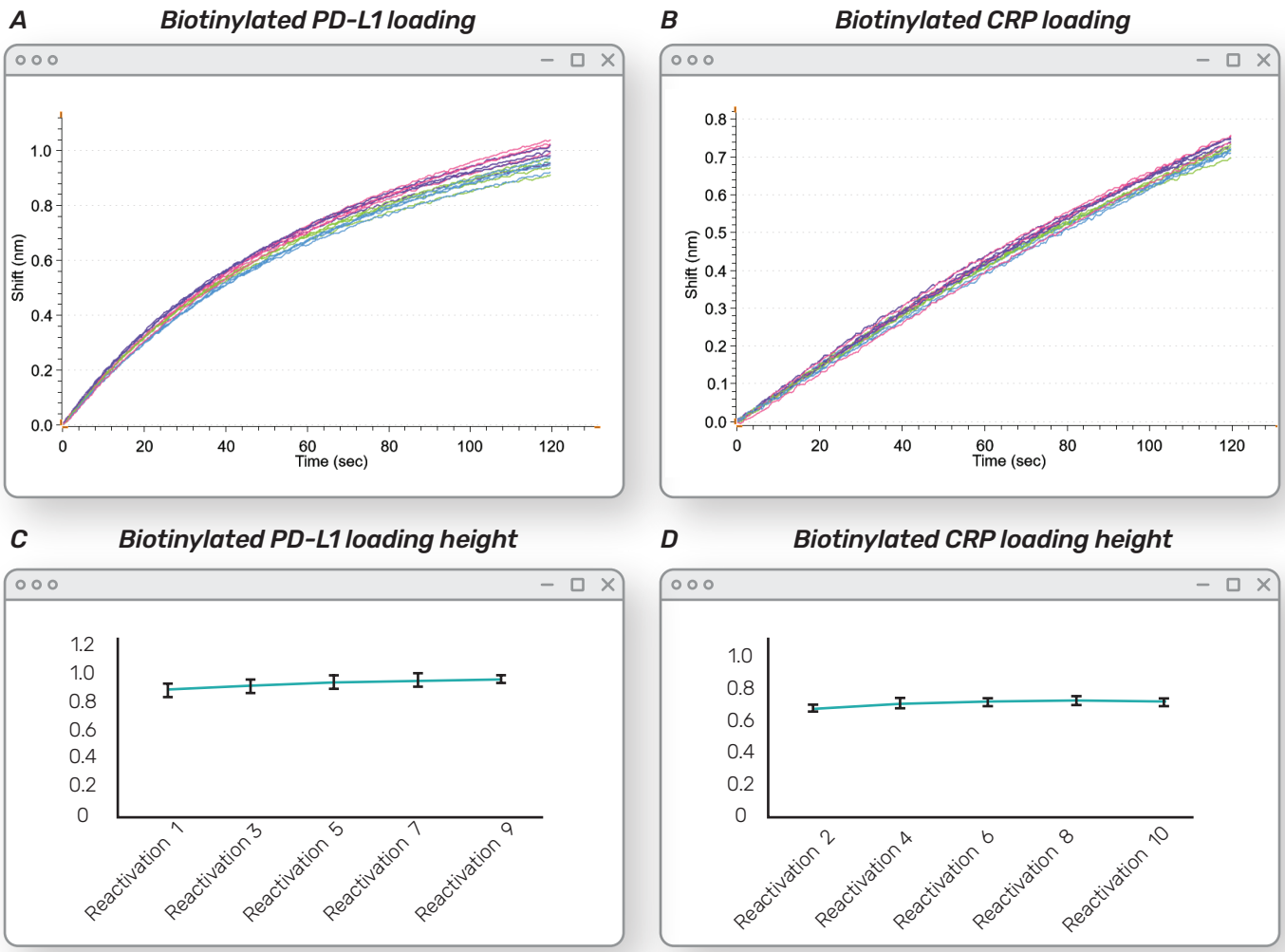


Figure 10. Alternating proteins using the Flex SA and reactivation. *Sensogram showing loading for PD-L1 (A) and C-reactive protein (B) over reactivations 1,3,5,7, 9 (C) and 2,4,6,8,10 (D). The loading signal for both proteins is consistent when loaded alternatingly after each reactivation.*

Conclusion

Streptavidin (SA) probes are one of the most useful and broadly applicable sensors for biolayer interferometry. Through innovations in optics and chemistry, Gator Bio now provides a selection of SA probes that extends the utility of BLI even further to tackle previously difficult or impossible applications.

Gator Bio's SA probes are capable of measuring molecules spanning many orders of magnitude in molecular weight. With the small molecule analysis probe (SMAP), kinetic measurements for small molecules as low as 150 Da can be performed. Alternatively, the SA XT probe enables binding studies for much larger macromolecules such as for lipid nanoparticles (LNPs) in the mega Da range. The SA XT probe also extends sensitivity, enabling measurements of very low concentrations of analyte and producing stronger signals overall for more accurate and reproducible binding and kinetics studies. With the Flex SA probe, Gator Bio addresses the need for more economical and regenerative multi-use sensors. Capable of up to 40 reactivations, and with no limitation on dedicating the probe to a single analyte, the Flex SA probe improves productivity and extends usability.

From affinity and competitive binding studies to binding kinetics and concentration determination, Gator Bio SA probes and BLI systems provide the power and versatility to not only meet the needs for conventional studies but also provide opportunities for generating fast and reliable data to support new applications across all stages of biotherapeutic research and development.

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