Evaluating the Versatility of Gator® Next Generation BLI Platform for Biotherapeutic Development and Gene Therapy

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Introduction

Biolayer Interferometry (BLI)

- 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 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 proteintherapeutics interactions. protein development and viral vector analysis
- Tolerant to different buffers, cell media, crude lysates, serum and plasma

High throughput Gator® Pro Instrument

- Fast, automated and accurate biomolecule characterization
- Built-in 32 spectrometers for up to 32 parallel biomolecule interactions in as little as 2 mins
- Three sample plates and one biosensor plate for high-throughput monitoring
- Flexible 96-well and 384-well sample plate format

Here, we present data from some unique applications like,

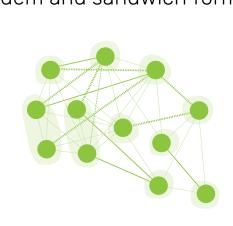
- Epitope binning
- Biosimilar kinetics
- LNP solutions
- Nanobody screening
- Small molecule interactions AAV solutions

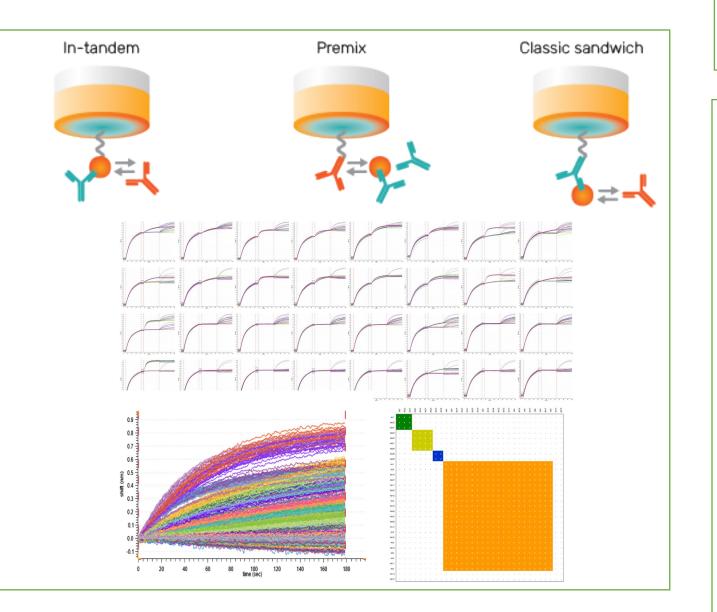
Gator® Bio's huge portfolio of biosensors can support at multiple stages of therapeutic development and in gene therapy.

Epitope Binning

- High-throughput 32 x 32 epitope binning assay utilizing Gator® Pro instrument in less than 8
- Accurate and automated tandem or traditional sandwich format
- Easy data visualization and
- Broad range of biosensors for tandem and sandwich format

interpretation

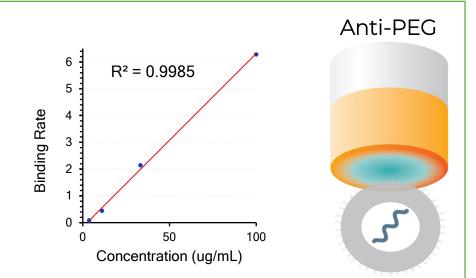




HFC II **Biosimilar Kinetics** Gator® Human Fc (HFC) Receptor II biosensor detects Fc region of all four IgG isotype making it suitable for biosimilar screening • The biosensor can be regenerated up to 20 times without loss in signal, thus making them cost effective • No cross-reactivity to Human Fab region and other species antibody, making them very specific Cetuximab: EGFR Matuzumab: EGFR Nimotuzumab: EGFR

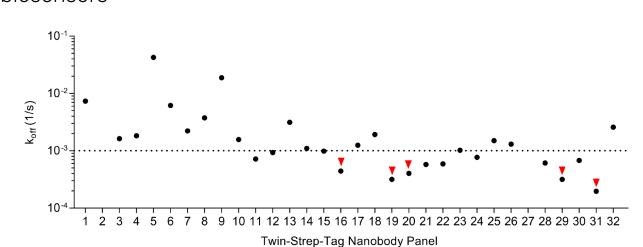
Lipid Nanoparticle Solutions

- Anti-PEG probes enables the detection and quantitation of LNPs
- Using unique optical layer, no inverted binding signal from LNP binding
- Serum proteins can be immobilized onto the probes to study the interaction with LNPs

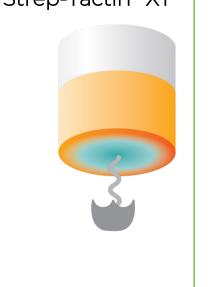


Nanobody Screening

- High affinity (pM) interactions between twin-Strep-tag fused protein, Fab fragments or nanobody with Strep-Tactin® XT biosensors
- Fast and accurate twin-strep-tagged nanobody screening using Strep-Tactin® XT biosensors



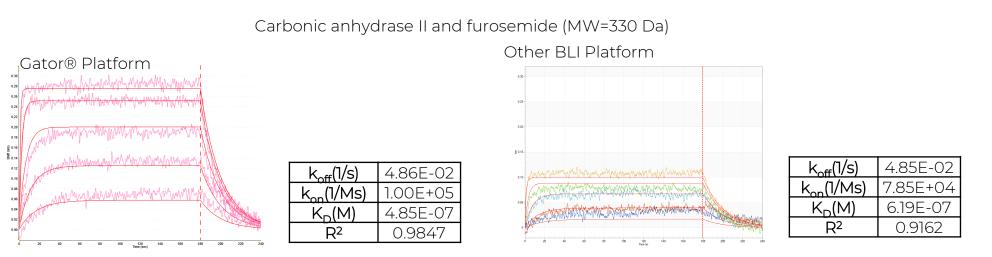
Strep-Tactin®XT



Small molecule interactions

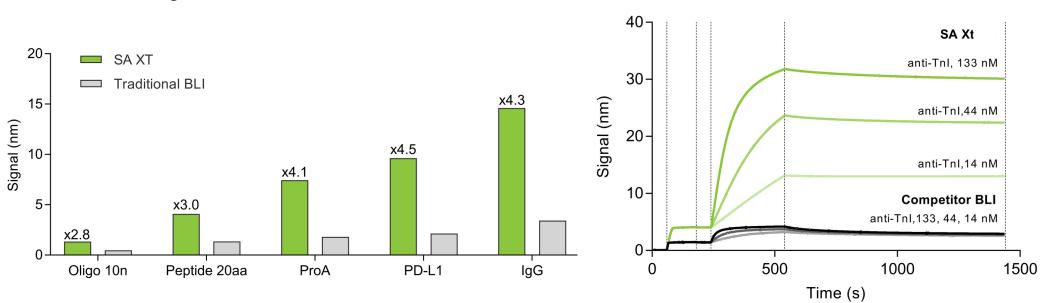
Gator® SMAP Probes

- Detect small molecules up to 150 Da with a binding partner
- Streptavidin based proprietary surface chemistry for high-capacity immobilization of biotinylated binding partner
- Determination of the kinetic parameters (k_{on} , k_{off} and K_D) of the small molecule with the immobilized binding partner
- Enhanced signals vs traditional BLI platforms



Peptide binding using Gator® Streptavidin (SA) XT Probes

- The biosensors detect biotinylated oligos, peptides and proteins above 1 kDa • Unique optical layer with novel proprietary chemistry enhances the signal 5-3x than the traditional BLI platform
- Large biomolecule up to 2 MDa can be detected without inversion of signal
- Higher signal allows for lower loading of ligand and analyte, hence conserving precious sample
- Accurate determination of the kinetic parameters (k_{on}, k_{off}) and K_D from small peptides to large biomolecules



AAV Solutions

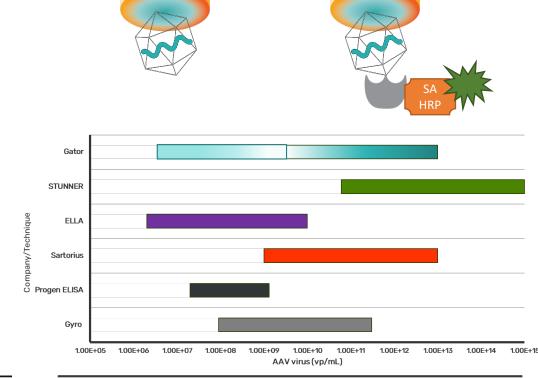
AAV capsid Titer in Crude Samples

- Gator® HS AAV/AAV9 kit is a "dilute and dip" method, perfect for upstream samples
- The kit accurately determines the AAV capsid titer without matrix interference
- The sensitivity is enhanced due to patented amplification technology
- Less hands-on time than ELISA

AAV capsid Titer in Purified Samples

 Together Gator® AAVX/AAV9 biosensors and Gator® High sensitivity (HS) AAV/AAV9 kit detects high dynamic range of AAV serotypes and recombinant AAV capsids (1.00E+07 to 1.00E+13 vp/mL)

Titer	5.33E+07 of <i>AAV</i> 5	1.00E+09 of <i>AAV8</i>		
Matrix Interference	% Recovery			
HEK293T Lysate* (~100 mg/mL of intracellular proteins from 1E+08 cells/mL of HEK293T cell suspended in PBS, 1:10 dilution in buffer)	80.18%	115%		
Cell Lysis Buffer (1X PBS, 100mM NaCl, 0.001% Pluronic)	96.31%	119%		
Spent Media (DMEM media, 10% FBS, 2mM L- Glutamine)	84.08%	110%		



High Sensitivity Assay

AAV/AAV9

DNA Quantitation

SMAP

SA-XT

5.33E+07 of <i>AAV5</i>	1.00E+09 of <i>AAV</i> 8		Gator®: HS AAV		PROGEN: AAV2 ELISA	
% Recovery			Crude Sample (collaborator samples)	Calculated Titer (vp/mL) N=2	Calculated Titer (vp/mL) N=1	
80.18%	115%		CS1	1.11E+12	OFR	
		CS2	1.46E+11	OFR		
96.31%	119%	CS3		3.63E+11	1.23E+11	
	11370	11570	CS4		3.80E+11	4.48E+11
84.08%	110%	CS5 4.1		4.12E+11	3.96E+11	
		CS6	3.88E+11	5.35E+11		

Lysis

AAV Capture

Direct Binding

AAVX/AAV9

Determination of AAV Empty vs Full Ratio

- Easy AAV Empty/Full ratio determination using Gator® AAV Ratio kit
- · The process involves AAV capsid capture, lysis followed by DNA quantitation
- Compatible with complex buffers and crude matrices
- Fast assay time without compromising
- accuracy

	% Difference between expected and measured values (collaborator samples)								$R^2 = 0.98$
% Full	Buffer 1	Buffer 2	Buffer 3	Buffer 4	Buffer 5	Buffer 6	Buffer 7	Buffer 8	# 20-
90	9%	4%	2%	14%	13%	18%	6%	10%	- 10-
75	12%	13%	3%	24%	18%	17%	8%	19%	0 50 100
60	2%	1%	15%	28%	4%	3%	13%	17%	% full
45	5%	12%	6%	19%	10%	0%	19%	19%	<15% difference form expected values
30	2%	22%	9%	12%	8%	8%	11%	12%	15-25% difference from expected value
10	27%	33%	40%	5%	9%	11%	38%	3%	>25% difference from expected value

Conclusion

- Gator® next generation BLI platform: one tool, many answers
- Fast biomolecule characterization using Gator® Pro Instrument
- Automated, accurate and fast epitope binning Precise and efficient biosimilar kinetics
- Accurate and easy kinetic platform for LNPs
- Easy and specific nanobody screening
- Enhanced small molecule and protein kinetic interactions
- Accurate kinetic parameters for small peptides to large protein using Gator® SA XT biosensors
- Total AAV solutions:
 - Precise AAV titer from upstream to downstream samples
 - Accurate determination of Empty vs full ratio
- Comprehensive suites of biosensors to support different development stages • Faster and cost-effective solutions in biotherapeutic development and gene therapy

