# Evaluating the Versatility of Gator® Next Generation BLI Platform for Biotherapeutic Development and AAV Analytics

Harsha Agarwal, Johnny Zhang, Wai Choi, Lu Peng, Indrani Chakraborty, Vivienne Lee
Gator Bio, Palo Alto, CA

## Introduction

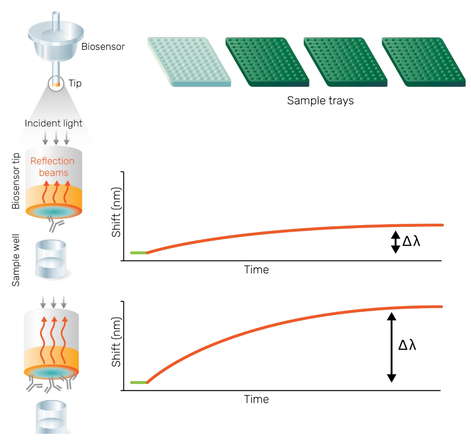
### Biolayer Interferometry (BLI)

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

Here, we present data from some unique applications such as,

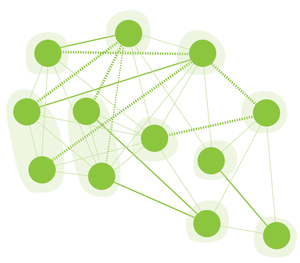
- Epitope binning
- Biosimilar kinetics
- LNP quantitation
- Nanobody screening
- Small molecule interactions
- AAV analytics

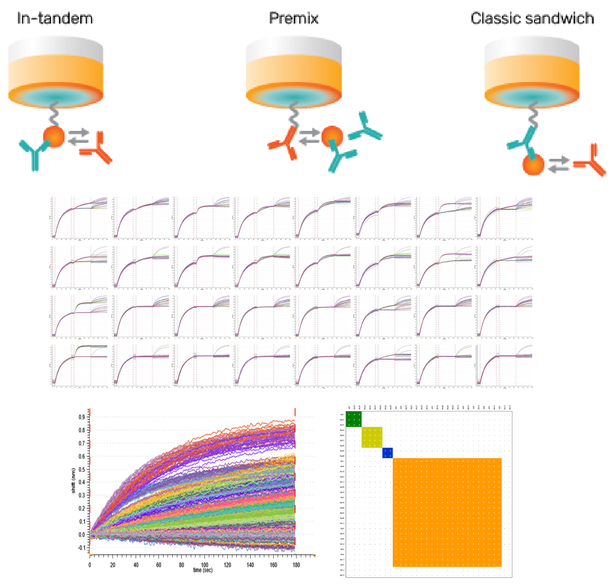
Gator® Bio's broad portfolio of biosensors can support at multiple stages of therapeutic development and in gene therapy.




## Epitope Binning

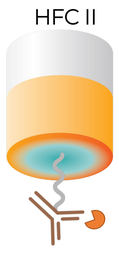
- High-throughput 32 x 32 epitope binning assay utilizing Gator® Pro instrument in less than 8 hours
- Accurate and automated tandem or traditional sandwich format
- Easy data visualization and interpretation
- Broad range of biosensors for tandem and sandwich format



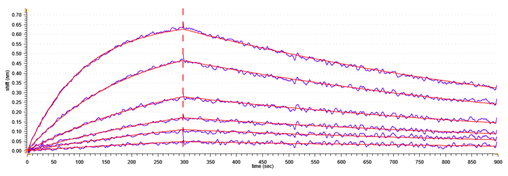



## Biosimilar Kinetics

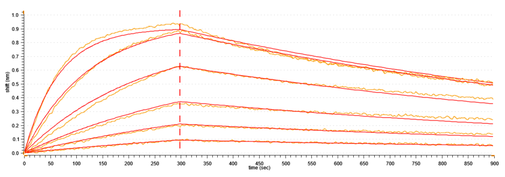
- Gator® Human Fc (HFC) Receptor II biosensor detects Fc region of all four IgG isotype making it suitable for biosimilar screening
- The biosensor can be regenerated up to 20 times without loss in signal, thus making them cost effective
- No cross-reactivity to Human Fab region and other species antibody,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



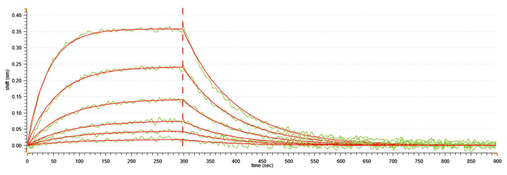

Cetuximab: EGFR



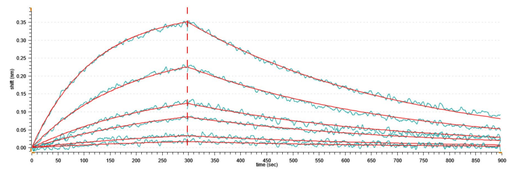
Zalutumumab: EGFR



Matuzumab: EGFR

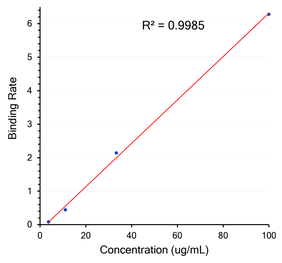


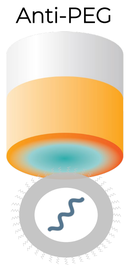
Nimotuzumab: EGFR



## Lipid Nanoparticle Quantitation

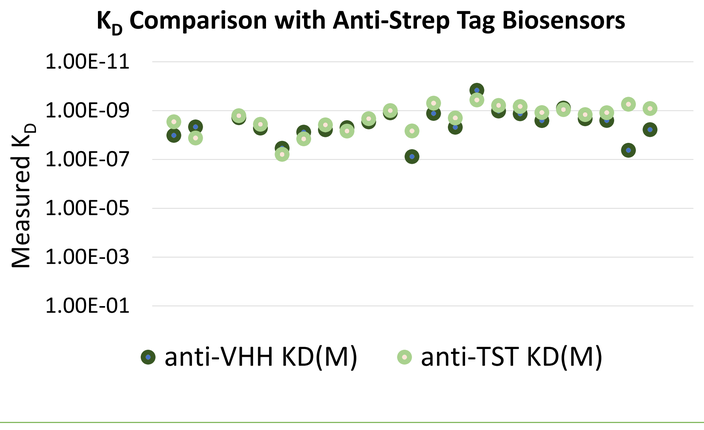
- Anti-PEG probes enables the detection and quantitation of LNPs
- Using unique optical layer, no inverted binding signal from LNP binding
- Serum proteins can be immobilized onto the probes to study the interaction with LNPs




## Nanobody Screening

- One-of-a-kind nanobody screening biosensor (Anti-VHH).
- Fast and accurate twin-strep-tagged nanobody screening using Strep-Tactin® XT biosensors.
- $K_D$ comparison studies of nanobody screening using both the biosensors shows comparable results.

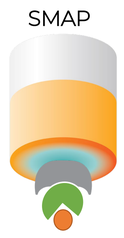




## Small molecule interactions

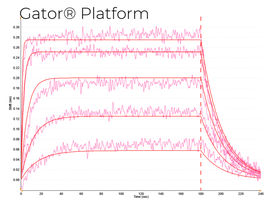
### Gator® SMAP Probes

- 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



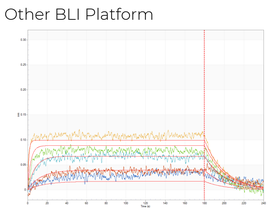

Carbonic anhydrase II and furosemide (MW=330 Da)

Gator® Platform



| | |
|---|---|
| $k_{off}$(1/s) | 4.86E-02 |
| $k_{on}$(1/Ms) | 1.00E+05 |
| $K_D$(M) | 4.85E-07 |
| $R^2$ | 0.9847 |

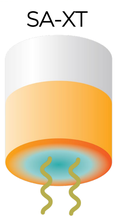
Other BLI Platform

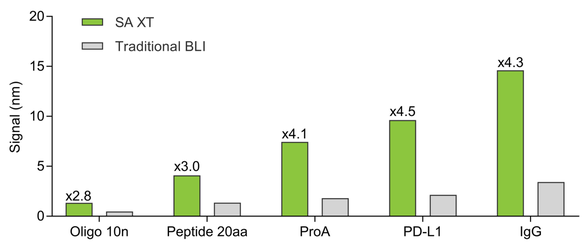


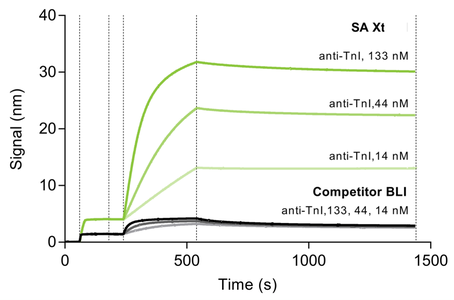
| | |
|---|---|
| $k_{off}$(1/s) | 4.85E-02 |
| $k_{on}$(1/Ms) | 7.85E+04 |
| $K_D$(M) | 6.19E-07 |
| $R^2$ | 0.9162 |

### 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 Analytics

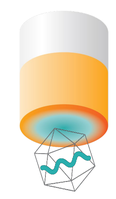
### AAV Capsid Titer in Crude Samples

- 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

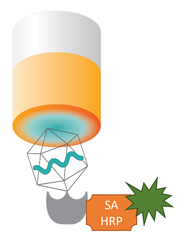
### AAV Capsid Titer in Purified Samples

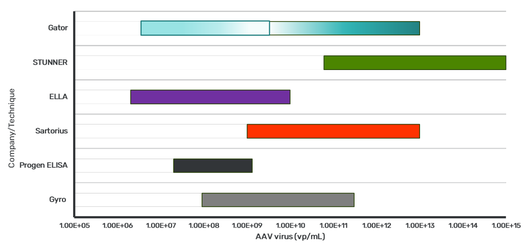
- Together Gator® AAVX/AAV9 biosensors and Gator® High sensitivity (HS) AAV/AAV9 kit detects high dynamic range of AAV serotypes and recombinant AAV capsids (1.00E+07 to 1.00E+13 vp/mL)

Direct Binding AAVX/AAV9



High Sensitivity Assay AAV/AAV9



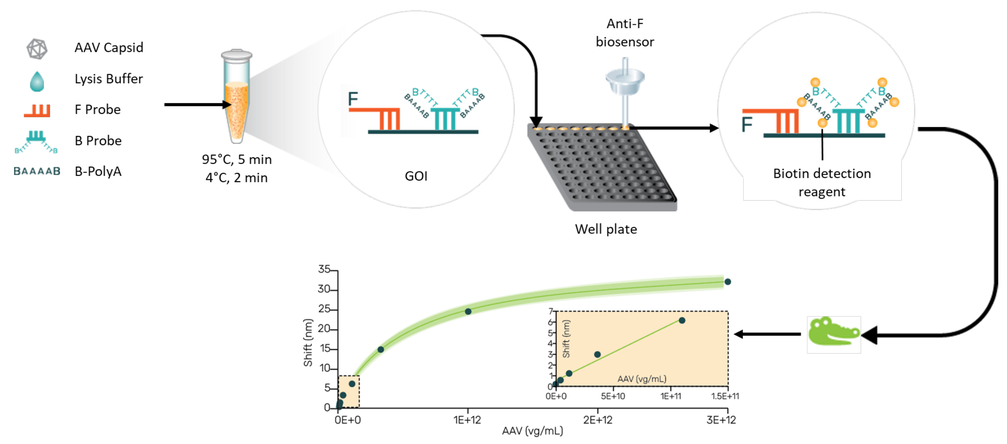




| Titer | 5.33E+07 of *AAV5* | 1.00E+09 of *AAV8* |
|---|---|---|
| Matrix Interference | % Recovery | |
| HEK293T Lysate* (~100 mg/mL of intracellular proteins from 1E+08 cells/mL of HEK293T cell suspended in PBS, 1:10 dilution in buffer) | 80.18% | 115% |
| Cell Lysis Buffer (1X PBS, 100mM NaCl, 0.001% Pluronic) | 96.31% | 119% |
| Spent Media (DMEM media, 10% FBS, 2mM L-Glutamine) | 84.08% | 110% |

| Crude Sample (collaborator samples) | Gator®: HS AAV Calculated Titer (vp/mL) N=2 | PROGEN: AAV2 ELISA Calculated Titer (vp/mL) N=1 |
|---|---|---|
| CS1 | 1.11E+12 | OFR |
| CS2 | 1.46E+11 | OFR |
| CS3 | 3.63E+11 | 1.23E+11 |
| CS4 | 3.80E+11 | 4.48E+11 |
| CS5 | 4.12E+11 | 3.96E+11 |
| CS6 | 3.88E+11 | 5.35E+11 |

### Determination of AAV Genome Titer

- Simple, fast and accurate determination of genome integrity
- The process involves hybridization and lysis in a single step
- Assay is very specific with high dynamic range
- No PCR involved




## Conclusion

- 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
  - Easy and fast detection of Genome titer

gator