AI-Engineered NovoBody™-Based Probe for “High Capacity, Low Drift” BLI Analysis of His-Tagged Proteins

Anuja Patil, Jacob Szymkowski, Vivienne Lee, Pu Li, Indrani Chakraborty

Abstract

We report performance characterization of the Gator® HIS XT probe, a bio-layer interferometry platform utilizing an AI-designed anti-His NovoBody™ for label-free analysis of His-tagged proteins. The NovoBody™ was generated through de novo computational design optimized for poly-His recognition, providing an engineered alternative to Ni-NTA chelation and polyclonal antibody approaches.

Comparative benchmarking demonstrated 5-fold expanded dynamic range (0.2–1000 µg/mL vs. 12.5–500 µg/mL for traditional probes), with post-loading baseline drift of 0.006 nm/min enabling high-resolution determination of slow dissociation rates. Orientation-independent capture of both N- and C-terminal His-tagged proteins showed consistent responses across 20 diverse targets. The surface maintained more than 90% binding capacity over regeneration cycles.

Kinetic analysis spanning pM to µM affinities produced high-quality fits (Chi² <1.0) comparable to SPR. Tandem epitope binning of 16 anti-TnI antibodies against His-tagged troponin I demonstrated clear discrimination between competitive and non-competitive pairs. The compact NovoBody™ scaffold (~32 kDa) delivers enhanced signal-to-mass ratios while eliminating Fc-mediated non-specific binding, demonstrating computational protein design as a strategy for generating analytical reagents tailored to BLI requirements.

Key Advantages of NovoBody™- based Gator HIS XT Probe

The Gator HIS XT biosensor features a NovoBody™ capture ligand engineered by Monod Bio specifically for high-performance His-tagged protein analysis on BLI platforms. Through AI-guided *de novo* protein design, the NovoBody™ was optimized to meet the unique detection requirements of bio-layer interferometry, resulting in a compact protein scaffold (~32kDa) that maximizes signal-to-mass optical response while eliminating Fc-mediated non-specific binding inherent to antibody-based capture surfaces.

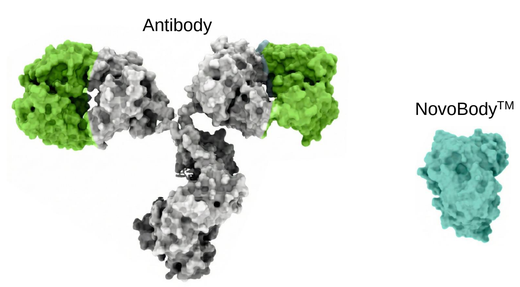


Figure 1: Antibody vs. NovoBody™

- ✔ Compact size delivers proportional optical response and high signal-to-mass ratio
- ✔ No Fc receptor non-specific binding; cleaner baselines and reduced matrix effects
- ✔ Universal capture of diverse His-tagged proteins regardless of tag position
- ✔ Reliable performance across multiple regeneration cycles and assay conditions

High Loading Capacity, Broad Dynamic Range

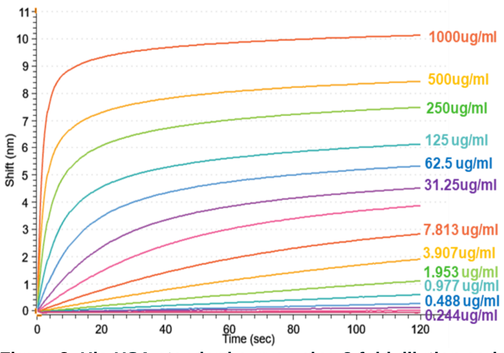


Figure 2: His-HSA standard curve using 2-fold dilution series from 1000 µg/mL to 0.24 µg/mL and measured using the Gator HIS XT biosensor with a 120 sec acquisition at 400 rpm.

The Gator HIS XT Probe features an advanced optical design that delivers a more proportional response relative to the molecular weight of captured proteins. This enhanced optical sensitivity produces high-magnitude signals even at significantly lower ligand concentrations, maximizing the signal-to-mass ratio.

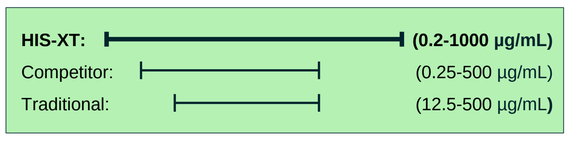
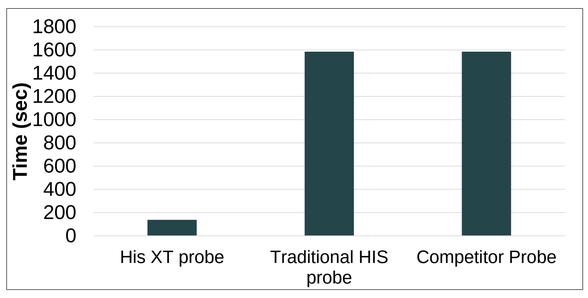


Figure 3: Gator HIS XT biosensor offers the broadest detection range (0.2–1000 µg/mL), spanning 5× wider than traditional His probes (12.5–500 µg/mL) and competitor probes (0.25–500 µg/mL) for superior quantitation flexibility

High sensitivity minimizes ligand consumption for cost-effective analysis

The Gator HIS XT Probe's superior signal-to-mass ratio enables high-quality quantitation and kinetic data while dramatically reducing consumption of precious His-tagged recombinant samples for cost-effective workflows.



- ✔ Strong loading signal with lower concentrations of his-tagged protein sample
- ✔ HIS XT achieves the target response in the shortest time as compared to other HIS probes
- ✔ Consistent performance across 20 His-tagged proteins tested

Figure 4: HIS XT achieves the target response in the shortest time using 1ug/mL his-tagged SARS-CoV-2 (2019-nCoV) Spike RBD-His Recombinant Protein, demonstrating faster capture kinetics than the other His probes.

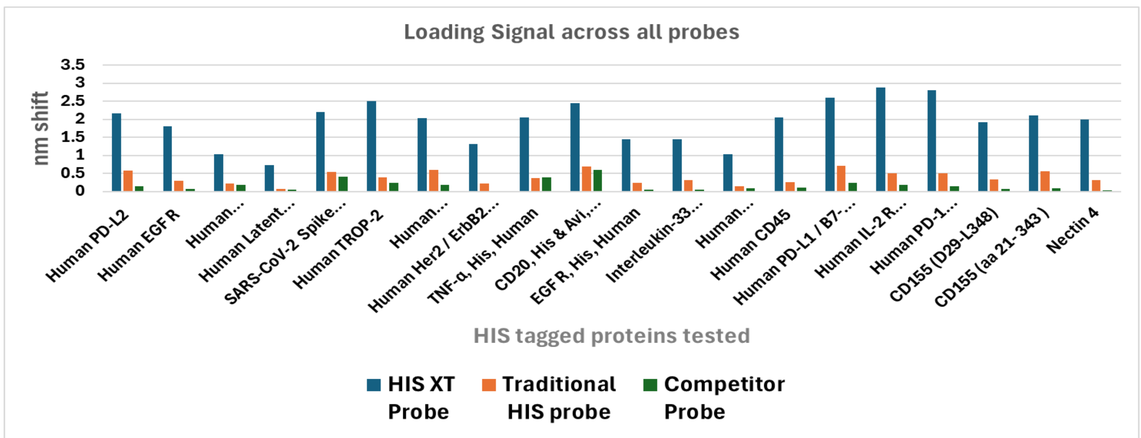
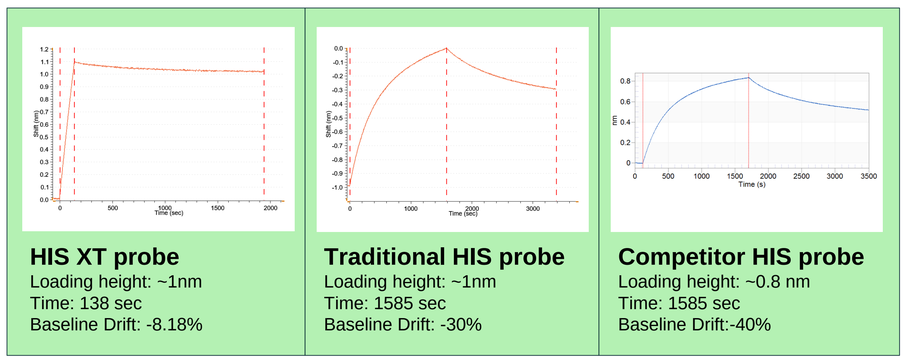


Figure 5: Gator HIS XT consistently exhibits high loading signals compared to traditional and competitor His probes when the same concentration of 20 diverse His-tagged proteins were tested.

High-Resolution Kinetic Analysis with HIS XT Probe

The Gator HIS XT Probe delivers high-resolution kinetic analysis of His-tagged targets by providing superior baseline stability, which is essential for accurate kinetic studies and minimizes data artifacts from drift. Its exceptional low baseline drift and high loading capacity produce cleaner sensorgrams with better fit quality, enabling precise determination of association/dissociation rates even for slow off-rates. Lower loading levels further reduce avidity effects compared to traditional His probes, while HIS XT 's optimized design improves overall data quality for protein–protein, protein–antibody, and protein–peptide interactions.



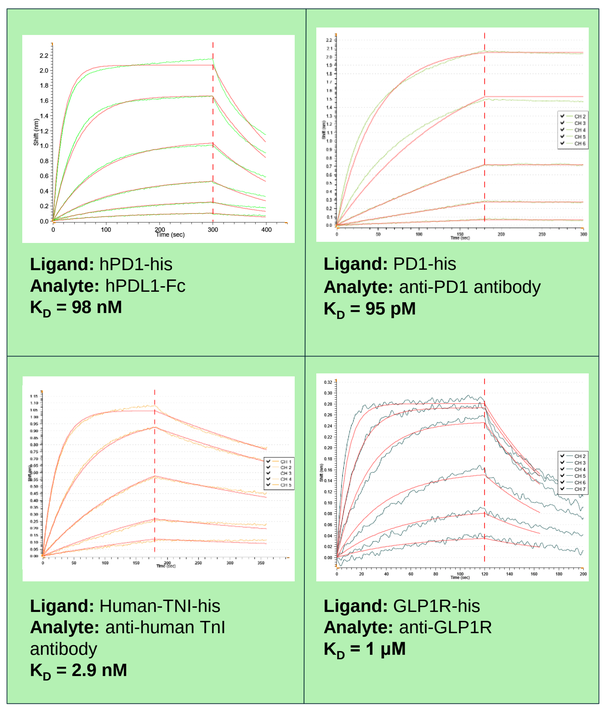
HIS XT probe
Loading height: ~1nm
Time: 138 sec
Baseline Drift: -8.18%

Traditional HIS probe
Loading height: ~1nm
Time: 1585 sec
Baseline Drift: -30%

Competitor HIS probe
Loading height: ~0.8 nm
Time: 1585 sec
Baseline Drift:-40%

- ✔ Minimal post-loading baseline drift supports high-resolution kinetic fits

Figure 6: All three His sensors were loaded to ~1 nM response with using 1ug/mL his-tagged SARS-CoV-2 (2019-nCoV) Spike RBD-His Recombinant Protein. HIS XT reached the 1 nM loading signal target (~0.8 nM for competitor probe) in the shortest time and showed less than 10% baseline drift after the loading step, indicating excellent stability for high-resolution kinetic analysis.



- ✔ High-quality kinetics for protein–protein, protein–antibody, protein–peptide interactions comparable with SPR data
- ✔ Broad affinity range: pM to mM
- ✔ Stable baseline for extended association/dissociation phases
- ✔ Superior fit confidence (Chi² < 1.0)
- ✔ Minimal drift (< 10%) enables reliable slow off-rates
- ✔ Robust regeneration with < 10% loss of signal
- ✔ Automated workflows for His-tagged panels

Figure 7: Example kinetic binding profiles using the Gator His XT Probe.

Epitope Binning using the HIS XT Probe

Epitope binning classifies antibodies based on competitive binding to overlapping or distinct epitopes on the same antigen, aiding antibody discovery and characterization. The Gator HIS XT Probe streamlines this process through direct, high-efficiency capture of His-tagged antigens.

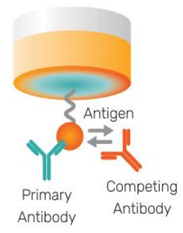


Figure 8: Tandem Epitope Binning

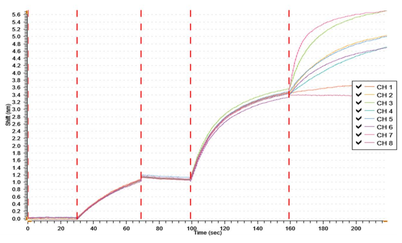
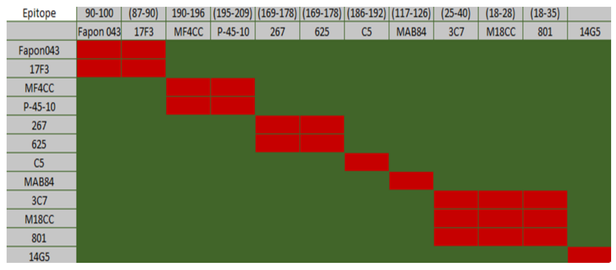


Figure 9: Representative sensorgram depicting Tandem Epitope Binning experiment



- ✔ High loading capacity and strong signal responses for clear binning readouts
- ✔ Stable baselines ensure reliable discrimination of competitive vs. non-competitive binding
- ✔ Optimized for tandem formats with sequential antibody binding to captured antigen

Figure 10: Tandem epitope binning analysis of a human TnI–His–tagged antigen against 16 anti-TnI monoclonal antibodies with known epitope diversity. In this assay format, the His-tagged antigen was first captured on the biosensor surface, followed by sequential binding of two antibodies (mAb1 and mAb2). The representative sensogram illustrates the reaction steps: (1) baseline, (2) antigen loading, (3) wash, (4) mAb1 association, and (5) mAb2 association. Figure 10 shows the corresponding heat map generated from the sensogram data, where red blocks indicate competitive antibody pairs recognizing the same or overlapping epitopes, and green blocks indicate non-competitive pairs binding to distinct epitopes.

Conclusion

The Gator HIS XT biosensor is the first commercial BLI platform utilizing a computationally designed NovoBody™ capture ligand. Developed with Monod Bio, this AI-engineered reagent delivers quantifiable performance improvements tailored to bio-layer interferometry requirements, including 5-fold expanded dynamic range (0.2–1000 µg/mL), minimal baseline drift 0.006 nM/min) for accurate slow off-rate determination, and high-quality kinetic fits (Chi² < 1.0) across pM to µM affinities. Orientation-independent capture was validated across 20 diverse His-tagged proteins in quantitation, kinetic characterization, and epitope binning workflows compatible with purified proteins and complex biological matrices.

Practical Advantages:

- ✔ More than 90% binding capacity over 10 regeneration cycles
- ✔ Reduced sample consumption via enhanced signal-to-mass ratio
- ✔ Consistent lot-to-lot performance for high-throughput discovery

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