Second-Generation Biolayer Interferometry as a Tool for Protein Engineering

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Introduction

Label-free analytical platforms are essential across the biologics workflow, enabling rapid, high-throughput measurement of titer and binding activity during cell line development (CLD), process development (PD), and quality control (QC). Data-Powered Therapeutics (DPTX) integrates cell-free expression of recombinant proteins with Gator Bio's second-generation biolayer interferometry (BLI) to provide purification-free, microfluidics-free, plate-based quantitation that seamlessly scales from discovery to regulated environments. Constructs bearing Twin-Strep tags are captured on Strep-Tactin® XT BLI probes and quantified against matrix-matched calibration curves, yielding time-resolved expression profiles (0–96 h) directly from crude supernatant. The same samples are then analyzed for label-free kinetics using purified binding partners in complementary assay orientations (ligand-on-probe or analyte-on-probe) under low loading and high agitation to minimize mass transport, allowing determination of k_{on}, k_{off}, and K_D through global fitting of 1:1 or heterogeneous models. Together, cell-free synthesis and second-generation BLI enable rapid, sensitive, and flexible analytics for construct screening, expression tracking, and kinetic characterization, accelerating data-driven decisions across biologics development (CLD→PD→QC).

- Real-time, label-free detection of biomolecular interactions
- Optical thickness change measured at the sensor during binding
- Proprietary probe chemistry to minimize non-specific binding
- High signal-to-noise from sensor and instrument design
- Direct use of crude samples (supernatants, lysates) without cleanup
- Accurate analysis without purification, supporting rapid construct triage and kinetics.

Methods

Microfluidics-free, plate-based BLI were used to quantify Twin-Strep-tagged constructs from crude matrices and to measure binding kinetics. BLI detects binding as changes in the optical thickness at the probe surface, enabling real-time, label-free readouts suitable for high-throughput screening.

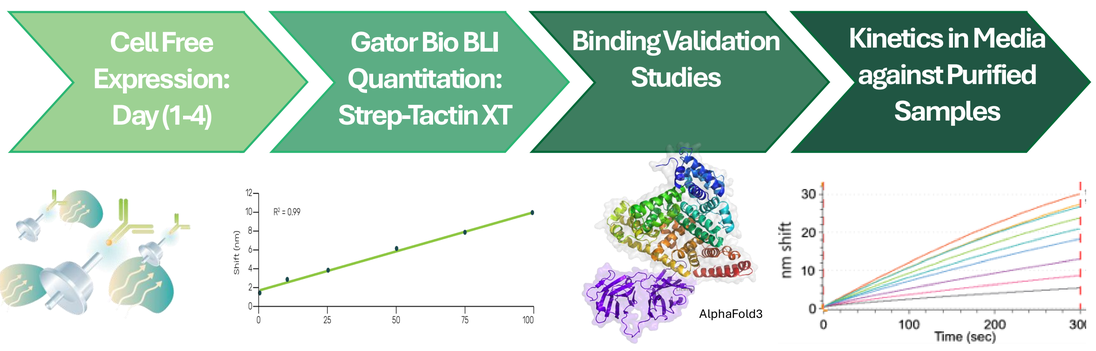

Figure 1. Microfluidics-free, plate-based BLI workflow for construct quantitation and binding validation. Data-Powered Therapeutics integrates (i) cell-free expression (Days 1–4; left) with (ii) Gator Bio BLI quantitation of Twin-Strep-tagged constructs directly from crude supernatants using Strep-Tactin® XT capture and matrix-matched calibration curves (middle plot). The workflow continues with (iii) label-free kinetic analysis in media against purified binding partners (right plot; representative sensorgrams) to assess affinity and specificity without purification. This fully plate-based, microfluidics-free approach enables high-throughput, real-time monitoring of expression yield and functional binding, with assays performed at low surface loading and controlled agitation to minimize mass-transport effects and support accurate kinetic modeling (k_{on}, k_{off}, K_D).

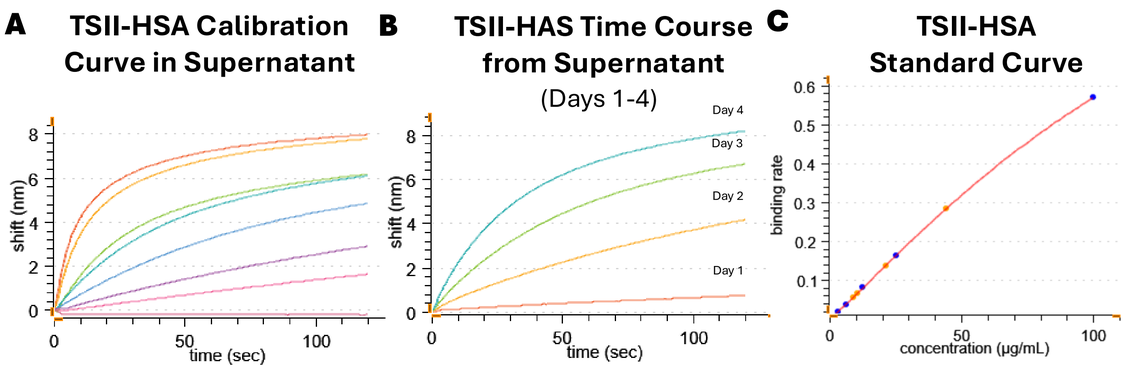

Figure 2. Time-course quantitation of TSII-HSA (Day 0–4) using Strep-Tactin® XT probes. BLI sensorgrams showing direct quantitation of HSA from crude supernatants. The first panel shows a 2-fold serial dilution standard curve of HSA from 100 µg/mL to 0 µg/mL. The middle panel presents Day 1–4 time-course data (n = 1 per day), demonstrating increasing HSA expression over time. The final panel displays the calibration curve (initial binding rate vs. concentration) used for conversion of binding response to concentration. All assays used Strep-Tactin® XT biosensors with matrix-matched calibration for consistent quantitation.

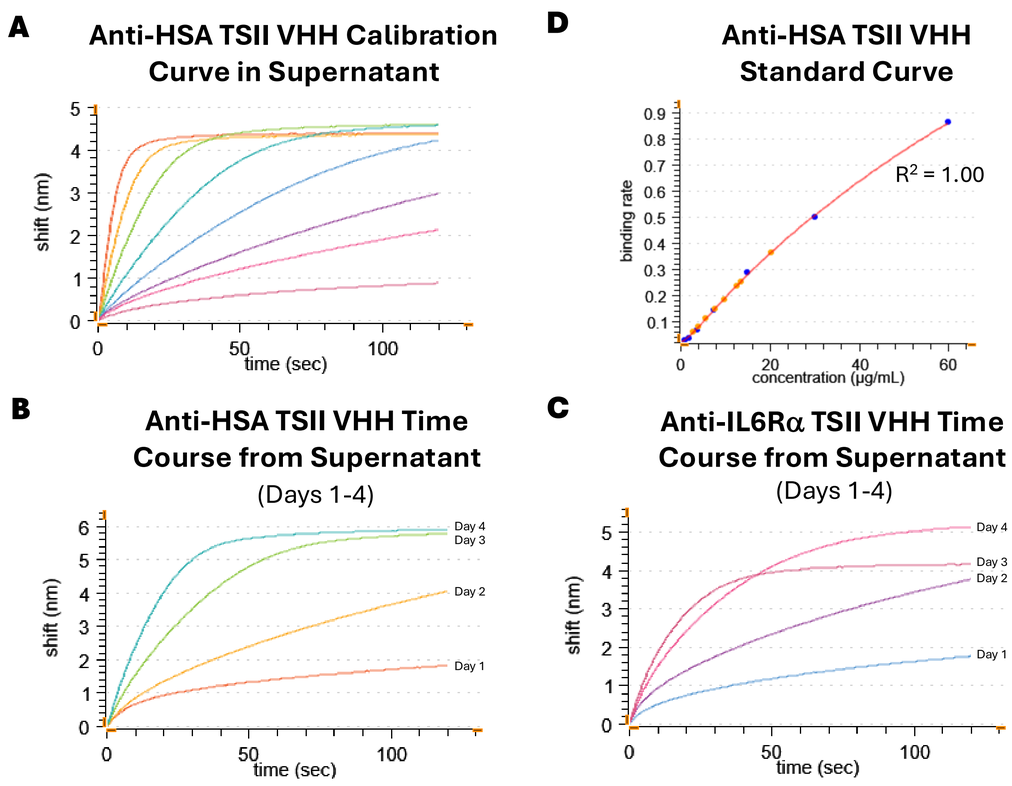

Figure 3. Time-course quantitation of anti-HSA TSII VHH (Day 0–4) using Strep-Tactin® XT probes. Direct BLI measurement of secreted anti-HSA TSII VHH from crude supernatants. The panel **(A)** shows a 2-fold anti-HAS Twin-Strep-tagged VHH standard curve (60 µg/mL to 0 µg/mL). Panels **(B-C)** show Day 1-44 sensorgrams of two VHHs showing increasing binding responses over time. Panel **(D)** shows the calibration fit converting nm shift to concentration, demonstrating consistent, label-free quantitation of VHH expression.

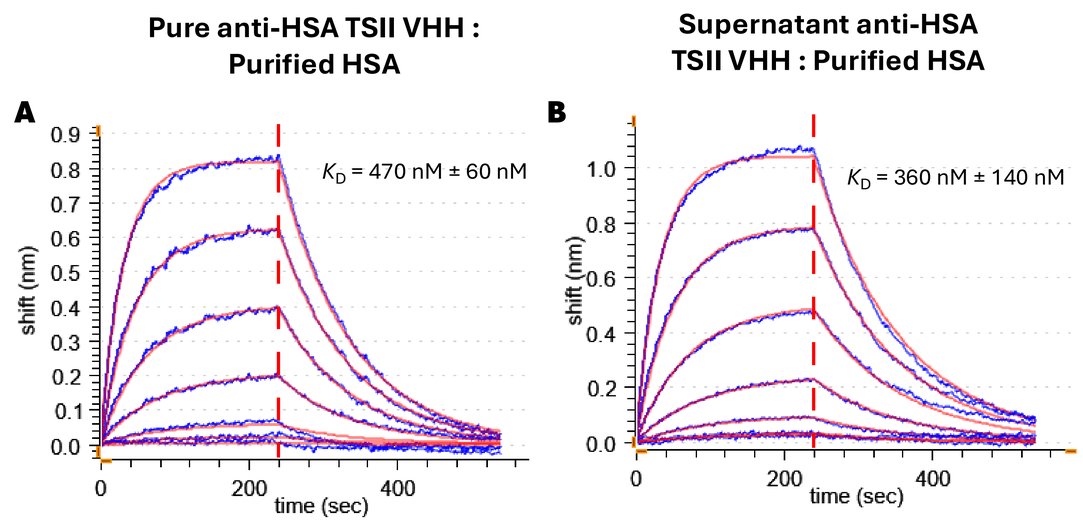

Figure 4. Kinetic analysis of VHH-HAS interactions from crude supernatants against purified analytes (Proteins A–B). BLI sensorgrams were recorded directly from clarified culture media containing Twin Strep-tagged ligands captured on Strep-Tactin® XT biosensors, followed by titration of purified analytes in PBS/HEPES + 0.05 % Tween-20 at 25 °C and 400 rpm. Ligand loading was minimized to achieve low response (R_{max}) values to reduce mass-transport and rebinding effects. Blue traces represent the raw binding responses, while red curves show global 1:1 kinetic fits. Vertical dashed red lines mark the transition between association and dissociation phases.

(A) Binding of purified anti-HSA TSII VHH to purified human serum albumin (HSA) analyte, yielding a K_D = 470 nM ± 60 nM (mean ± SD, n = 2).**(B)** Binding of anti-HSA TSII VHH directly from supernatant to its purified HSA, showing a K_D = 360 nM ± 140 nM.

Data were processed with reference and double-reference subtraction to remove nonspecific background and buffer artifacts. These results demonstrate that reliable kinetic constants can be obtained directly from crude expression media using proper immobilization and fitting strategies.

Conclusions

- **Collaboration with Gator Bio BLI:** In partnership with Gator Bio, DPTX applies data-driven analytical strategies to accelerate biologics characterization using second-generation biolayer interferometry (BLI).
- **Direct quantitation**: Strep-Tactin® XT probes capture Twin-Strep-tagged constructs directly from crude supernatant, providing time-resolved titers (Day 1 - 4).
- **Kinetic analysis**: The same samples are used for label-free kinetic measurements against purified partners, delivering precise k_{on}, k_{off}, and K_D values that inform construct performance and stability.
- **Workflow efficiency**: High-throughput, plate-based, microfluidics-free assays accelerate construct screening from discovery through CLD, PD, and QC.
- **Sensor reusability:** Strep-Tactin® XT sensors are regenerable, reducing assay cost and improving efficiency.