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Conversion of a viral glycoprotein titer method from ELISA to BLI

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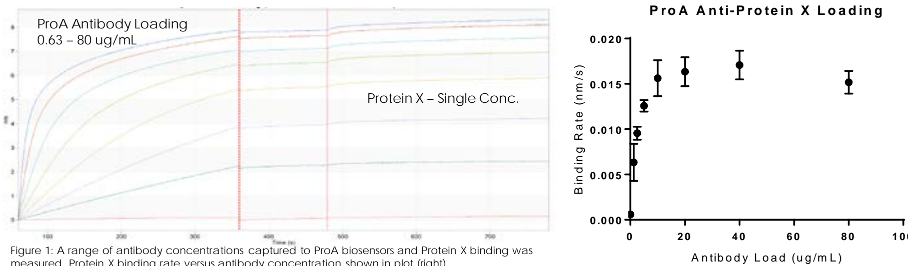
Abstract

Titer methods are useful at many stages during biopharmaceutical development from cell-line development to in-process control testing. Antibody-based titer methods can have the added benefit of recognizing properly folded and active forms of the biomolecule thus monitoring a critical quality attribute from bioreactor to drug substance. In this case study the Analytical Development group at Cytovance Biologics was provided an ELISA titer method and the method was redeveloped to operate by biolayer interferometry (BLI) using the ForteBio Octet RED96. The biomolecule of interest was a viral surface trimeric glycoprotein (Protein X) with a commercially available monoclonal antibody. Method development experiments involved testing antibody immobilization techniques, antibody loading conditions, standard curve and sample detection conditions. The developed BLI method was fully automated after plate preparation and approximately two times faster than the original ELISA method. The simplicity of experiment setup and analysis allowed bioreactor titer sampling to be performed by members of the upstream process development group. The method was qualified using guidance from ICH Q2R1 and has been transferred to the Quality Control group for titer testing of GMP samples.

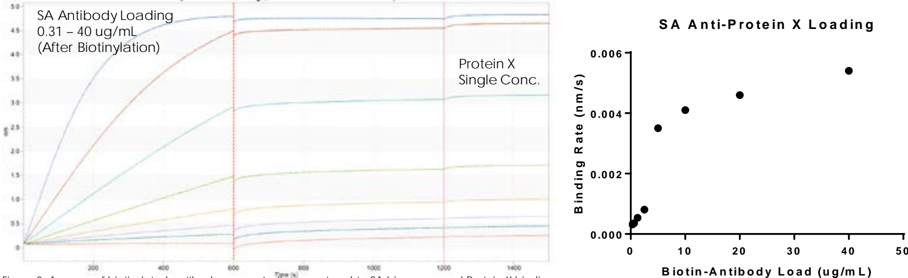
Method Development Parameters

BLI functions by first immobilizing or loading one molecular binding partner to the fiber optic biosensor and then measuring binding of the analyte. Loading conditions and biosensor selection are common development starting points. Biosensors functionalized with Protein A (ProA) or Streptavidin (SA) were tested for loading anti-Protein X at a range of concentrations. Subsequently the loaded biosensor was tested at a nominal concentration of Protein X. ProA biosensors loaded with antibody at 10 ug/mL gave the optimal loading condition with the highest binding rate of Protein X with the lowest concentration of antibody.



ProA Anti-Protein X Loading
Binding Rate (nm/s) vs. Antibody Load (ug/mL)

Figure 1: A range of antibody concentrations captured to ProA biosensors and Protein X binding was measured. Protein X binding rate versus antibody concentration shown in plot (right).



SA Anti-Protein X Loading
Binding Rate (nm/s) vs. Biotin-Antibody Load (ug/mL)

Figure 2: A range of biotinylated antibody concentrations captured to SA biosensors and Protein X binding was measured. Protein X binding rate versus antibody concentration shown in plot (right).

Standard Curve Optimization

Conditions for the Protein X standard curve were tested including the composition of sample diluent, Protein X concentration range, temperature, time and orbital shaking speed. Finally the potential for regenerating the biosensor tips with a harsh buffer condition was tested to enable reusing biosensors for multiple sample reads within a run. The optimized Protein X standard curve (0.8 – 100 ug/mL) was diluted in a sample diluent containing 1X PBS, 0.05% Tween-20 and 0.5% BSA and tested at a plate temperature of 30°C and orbital shake speed of 1000 rpm. Antibody loading was performed for 7 min followed by a 3 min wash in sample diluent and a 1 min read in the standards. Regeneration of biosensors was possible using 10 mM Glycine pH 1.5. The figures below show the optimized Protein X standard curve series versus time and versus concentration.

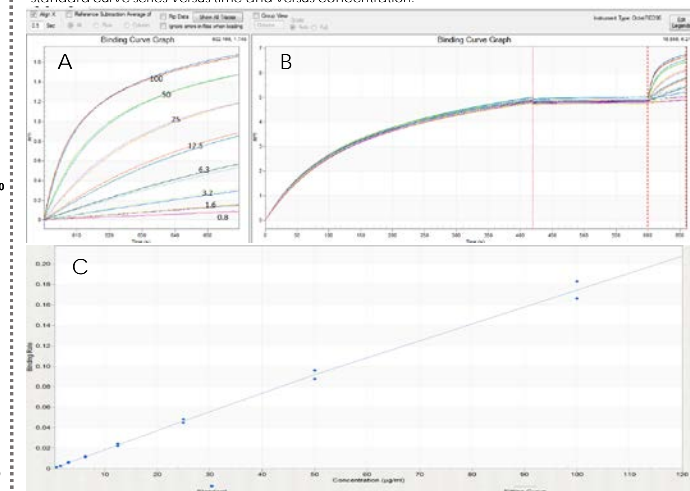


Figure 3: Protein X standards sensorgrams (A), full-scale experiment for Protein X standard sensorgrams (B), and binding rate versus Protein X concentration standard curve (C).

BLI Titer Workflow

Prepare Octet Sample Plate
- 30 Min
- Up to 16 samples

Load Plate

Automated Assay in Octet RED96
- 1.5 Hours
- Total Time to Result ~2 Hours
- Hands-On Time: 30 Min

Original ELISA Titer Workflow

Sample – Coat Plate
- 1 Hour or
- Overnight 4°C
- Up to 24 samples

Wash

Block Plate
- 1 Hour or
- Overnight 4°C

Wash

Add Primary Antibody
- 1 Hour

Wash

Add Secondary Antibody
- 30 Min

Wash

Add Substrate and Stop Solution
- 15 Min
- Total Time ~4 Hours
- Hands-On Time: 4 Hours

BLI Titer Method Advantages

- Faster time to results than the ELISA method with minimal hands-on time
- Ease of operation – process associates easily able to perform and monitor bioreactor titers
- Real-time data collection – aids investigations if aberrant results are obtained
- No plate washing required

Conclusions

The BLI-based titer method for the viral glycoprotein, Protein X, was developed with a quantifiable range of 0.78 – 100 µg/mL Protein X using a functional epitope binding antibody. The method uses the Octet RED96 system with pre-functionalized Protein A biosensors. The biosensors are regenerated after each read of 8 wells and including replicates and reagent wells up to 16 samples can be measured in one 96 well plate. The method was successfully transferred to the QC department after qualification and while in R&D was easily performed by associates in the process development group. The titer method will be used in ongoing manufacturing runs and to support stability programs for Protein X.

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