# High Throughput Bio-Layer Interferometry in Therapeutic Antibody Discovery and Development

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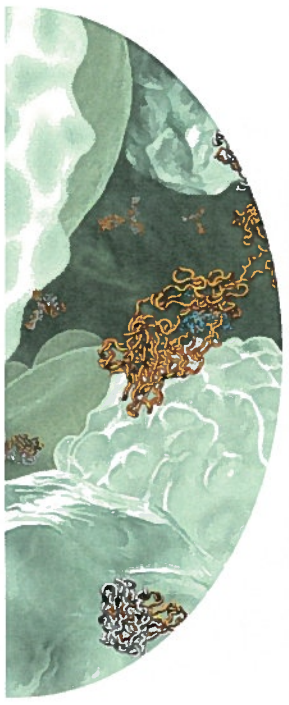


## Introduction

Bio-Layer Interferometry (BLI) plays an important role in the antibody discovery and development processes at Genmab. Integrating the quantification, kinetic and affinity output of our ForteBio Octet BLI platform with the data analysis capabilities of ActivityBase XE enables us to perform "high value" screening within early antibody discovery.

We have succeeded to significantly increase the throughput of label-free assays and accommodate more projects by implementing and integrating the new automation capable Octet RED384 instrument to a Tecan Freedom Evo workstation. Here we present first HTS BLI data on IgG quantification and affinity ranking timely integrated in early stages of the discovery process. Focusing on the use of recyclable biosensors the effects of sample impurities such as serum proteins and variations in sample concentration occurring during early stages of the discovery process are addressed. Further applications of high throughput BLI on the antibody discovery and development processes are discussed.

## Process Flow Automation

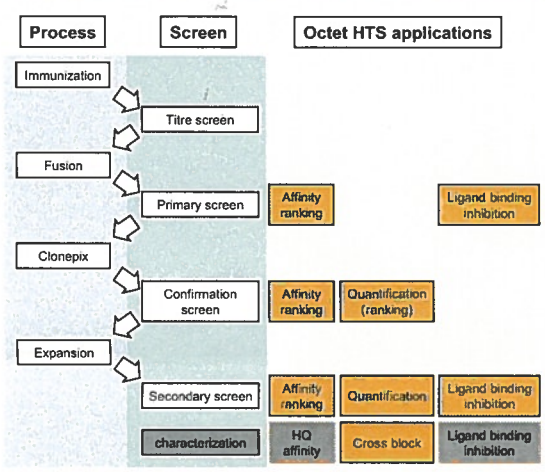
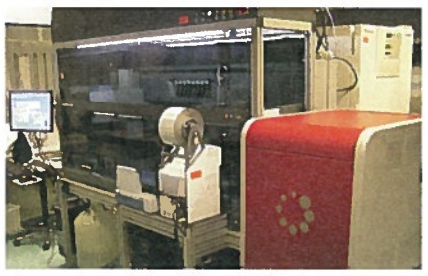



**Figure 1:** Applications of automated Bio-Layer Interferometry in antibody discovery using the Octet 384RED integrated with a Tecan Freedom Evo workstation.
**Chart:** An increased sample throughput through automation of the Octet384RED allows the integration of HTS BLI in 384-well format at early steps of antibody discovery including IgG quantification, affinity ranking, and Ligand Binding Inhibition assays. Typically, early process steps are hallmarked by low antibody concentrations and complex sample composition (eg. serum containing cell culture medium).



**Picture:** The Octet 384RED integrated to a Tecan Freedom Evo workstation via a docking station. The operation of the Ocet 384RED is controlled by the Tecan EVOware software. The Tecan integration enables inter alia the tray-wise (96 biosensors simultaneously) off-line regeneration of Protein A biosensors and the off-line coating of Amine Reactive biosensors using the Tecan TE-shake platform, thus streamlining label-free analysis.

## IgG Quantification

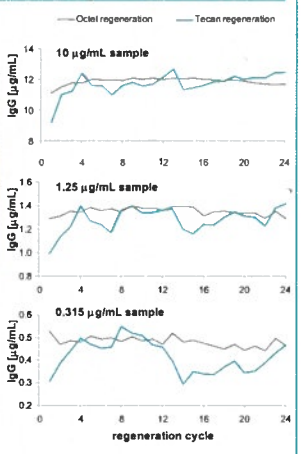



**Figure 2:** Off-line Tecan regeneration of Protein A biosensors for IgG Quantification.
**Left picture:** Tray with 96 biosensors positioned by the RoMa arm over a deep-well plate on the TE-shake platform during Protein A biosensor regeneration.
**Right chart:** Protein A biosensor performance in function of regeneration cycles. Comparing Octet on-line regeneration (maximal 16 biosensors simultaneously) with Tecan off-line regeneration (up to 96 biosensors simultaneously). IgG samples at three different concentrations in serum containing Hybridoma cell culture medium were repetitively quantified with Protein A biosensors regenerated either on-line (Octet) or off-line (Tecan). Optimal sensor performance was observed with 3 to 24 cycles of regeneration.

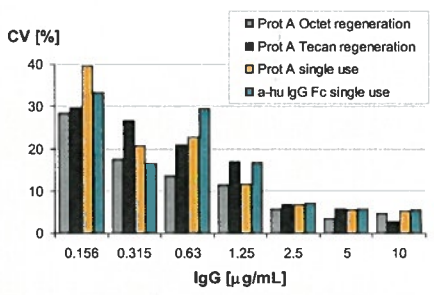



**Figure 3:** Qualification of BLI IgG concentration ranking methods for serum containing Hybridoma cell culture supernatant. Randomly distributed samples with process relevant IgG concentrations were repetitively quantified using either (i) on-line Octet regenerated Protein A biosensors, (ii) off-line Tecan regenerated Protein A biosensors, (iii) single use Protein A biosensors, or (iv) single use anti-human IgG Fc biosensors. CV values over 24 quantifications in duplicate with alternating sample concentrations are plotted. Protein A biosensors were cycled 24 times for regeneration including 3 cycles of pre-regeneration.

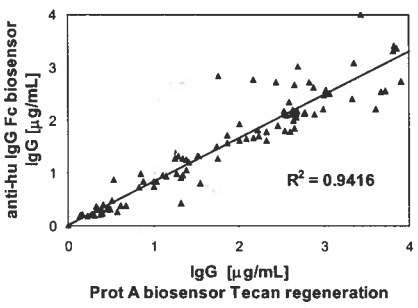



**Figure 4:** IgG concentration ranking with Tecan regenerated Protein A biosensors compared to single-use anti-human IgG Fc biosensors. The IgG production levels of 288 Hybridoma clones from Clonepix (serum containing cell culture supernatants) were determined with either single-use anti-human IgG Fc biosensors or Tecan regenerated Protein A biosensors. About one third (n=93) of the analysed Hybridoma clones yielded IgG production levels above a threshold of 0.1 µg/mL. Anti-human IgG Fc and Protein A biosensor data were plotted against each other excluding samples with more than 4 µg/mL IgG.

## Affinity Ranking

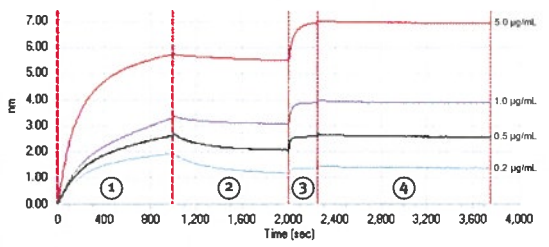



**Figure 5:** Sensorgrams of kinetic analyses at various antibody concentrations. Various concentrations of an antibody in serum containing Hybridoma cell culture medium were analysed with Protein A biosensors. Analysis steps: (1) Antibody binding, (2) Wash/ Baseline, (3) Antigen Association, (4) Antigen Dissociation. An extended wash/ baseline step (2) was implemented to wash off medium components unspecifically bound to the biosensor during the initial antibody binding step (1).

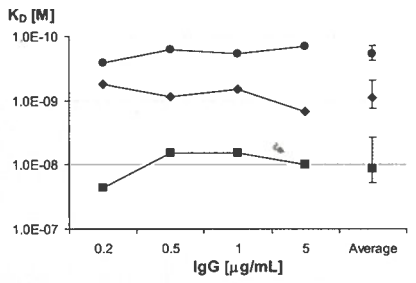



**Figure 6:** Dissociation constants in function of IgG sample concentration - Affinity ranking of early discovery stage antibody samples is not affected by sample concentration variation. Three antibodies with different dissociation constants (●, ♦, ■) were diluted to defined concentrations in serum containing Hybridoma cell culture medium (similar to Figure 5). Adjusted antibody end concentrations reflect relevant concentrations at early discovery stages. Dissociation constants of these antibody dilutions were determined using Protein A biosensors.

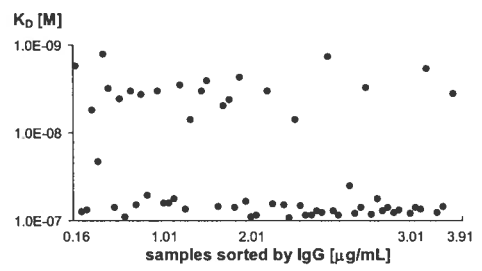



**Figure 7:** Automated affinity ranking of Hybridoma cell culture supernatant samples. Clonal Hybridoma samples at Clonepix stage with IgG concentrations ranging from 0.16 µg/mL to 3.9 µg/mL (from Figure 4) were subjected to automated Affinity Ranking with Protein A biosensors. 71 out of 93 IgG containing samples exhibited binding to the target antigen with affinities greater than 10-7 M. The separation of the samples into populations with different dissociation constants might reflect that the analysed samples were obtained from subclones of a limited number of parental Hybridomas.
A generic process would involve hit-picking from a primary or confirmation HTS screen and integrated hit transfer to automated HTS affinity ranking.

## Methods

**Buffers and sample preparation.** All samples were in Hybridoma cell culture supernatant or in Hybridoma cell culture medium containing 10% CCS. Sample diluent buffer: 138 mM NaCl, 2.7 mM KCl, 1mg/ml BSA, 0.02% Tween, 0.05% Sodium Azide (pH 7.4).
**Protein A biosensor regeneration.** On-line (Octet) regeneration: up to 16 biosensors were simultaneously regenerated by three alternating cycles of stripping for 5 sec in 10 mM Glycine pH 1.0 and neutralization for 5 sec in sample diluent buffer (shake rate 1000 rpm). Off-line (Tecan) regeneration: up to 96 biosensors were simultaneously regenerated following the on-line regeneration protocol (shake rate Tecan TE-shake platform 1000 rpm).
**IgG Quantification.** 100µL sample in 384 well plate. Shake rate 1000 rpm. Temperature 30°C. IgG binding to Protein A biosensors or anti-human IgG Fc biosensors was recorded for 120 seconds. Single use Protein A biosensors were pre-regenerated with 3 regeneration cycles before analysis; single use anti-human IgG Fc biosensors were pre-wetted in sample diluent for at least 5 min before analysis. Data processing and quantification was executed with ForteBio Data Analysis v6.2 software using a linear point-to-point standard curve and referencing to Hybridoma cell culture medium.
**Kinetic Analysis.** All kinetic analysis on this poster was performed with pre-wetted Protein A biosensors. No significant difference of kinetic data was observed when using anti-human IgG Fc biosensors instead of Protein A biosensors (data not shown). 100 µL sample in 384 well plate. Shake rate 1000 rpm. Temperature 30°C. Antibody binding was performed over 1000 sec followed by a wash/ baseline step of 1000 sec. Antigen association was monitored for 250 sec at four antigen concentrations. Antigen dissociation was monitored for 1500 sec. Data processing was executed with ForteBio Data Analysis v6.2 software using a global analysis on Rmax unlinked by sensor.

## Conclusions

- We implemented automated BLI analysis in our antibody discovery process by integrating the Octet 384RED with a Tecan Freedom Evo workstation.
- Automation of the Octet 384RED allows high throughput BLI analysis with interlacing work protocols including in particular Tecan driven bulk off-line biosensor regeneration and preparation.
- We successfully implemented IgG Concentration Ranking and Affinity Ranking in the presence of serum and other cell culture medium impurities with antibody panels varying 50-fold in concentration.
- Implementation of recyclable Protein A biosensors for IgG Concentration Ranking and Antibody Affinity Ranking at very early stages of our antibody discovery process reduce assay costs significantly (up to 20-fold).
- We are currently exploring the scale-up of further BLI applications and their implementation in "high value" screening within early antibody discovery including Ligand Binding Inhibition and Cross-Block assays.
- Bulk off-line Tecan coupling of proteins to Amine Reactive or Streptavidin biosensors allows HTS BLI assay development with customized biosensors.