Towards an optimized in-vitro SPR assay for antibody Fcγ receptor binding kinetics

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Introduction
Therapeutic antibodies are now approved for a number of different indications. One important antibody IAB attribute is its Fc effector function, and Abs are now being engineered with the aim of obtaining desired binding of the Ab Fc region to Fc receptors (FcR) on immune effector cells. Optimized and standardized Fc assays may impact the understanding of the function Fc-R interactions. They can potentially be used through all stages of antibody development, process development and QC and may also play an important role in comparability studies and for the evaluation of biosimilars.

The use of Biacore™ as a biochemical assay for studies of Fc γ Fc-R binding is already established in this area; however there is a lack of consensus regarding assay set up and evaluation of the often complex binding kinetics. Examples of published assay setups include amine coupling of FcR (1), anti-His capture of His tagged FcR (2), omme coupling of Ab (3), Protein A capture of Ab (4), anti-Fab capture of Ab (5). In the present study assay setups and kinetic evaluations using Biacore T200 are discussed. Results using commercially available, recombinant extracellular FcRs from two different expression systems, HEK293 (Sino Biologics) and NS0 cells (R&D Systems), are presented.

Kinetics depend on assay setup
Rituximab binding to Fcγ receptors
After feasibility studies of most of the published Biacore assay setups we focused on and compared two formats, with the aim of finding a setup suitable for screening of Ab – FcγR binding. Protein capture of Ab was compared more closely with anti-His MAb capture of His-tagged FcR.

His FcγR capture method development
The His FcγR capture approach was selected and investigated further in combination with Single cycle kinetics. The advantages over other assay set-ups were:
• The product is in solution, retaining full flexibility.
• Binding Protein A is possible for risk of binding site with Fab part (5).
• Ab is more often than FcR available in the high concentrations needed to measure small interations.
• Oriented capture of his tagged FcγR. Neutral buffer for immobilisation.
• Single cycle kinetics consumes low amounts of FcR.
• Easy retrieval of all FcγR.
• His capture kit convenience.

Selected conditions for these FcγRs

Specificity
To check for specificity and ensure that the His-tagged FcR do not bind to the Fc region of the immobilized mouse IgG1 anti-FcγR antibody, the anti-His mAb and the relevant mouse IgG1 antibodies were first introduced to the chip. Eight different binding models were then generated.

• Significant differences in binding to FcγRIIa was observed for IgG3 from Sigma revealed a heterogeneous collection of N-glycans based on the values of the constants complex 1 has a high affinity binding site which is smaller and the second complex a half life of 23 s.

Conclusions

• A His FcγR capture assay was found suitable for antibody-FcγR binding studies.
• Antibodies exhibited complex binding to both FcγRI and FcγRIIa receptors.
• Binding data indicated different binding mechanisms to FcγRI and FcγRIIa respectively.
• Binding to FcγRI could be modeled as two independent reactions while binding to FcγRIIa could be modeled by assuming two states of the reaction.
• Similar antibody binding kinetics was obtained comparing FcγRs from NS0 and HEK293 cells.
• Significant differences in binding to FcγRIIa was observed for IgG3 preparations with differences in glycosylation pattern.

References

Results
Antibody binding to Fcγ receptors from different cell lines

IgG3, with different glycosylation patterns, binding to FcγRIIa
To apply the His FcγR capture approach we aimed to replicate published data (1) for binding of human IgG antibodies to Fcγ. However, our data distinct agree for IgG3. One difference with our study was the assay setup, where another difference was that our IgG1 subclasses were obtained from Sigma while the published results were obtained using igg3 from The Binding Site.

Fig. 1. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 2. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 3. Single cycle kinetics analysis showing the binding between Rituximab and two commercially available anti-His mAbs. Single cycle kinetics were used to analyze binding of Rituximab to two different anti-His mAbs. The two anti-His mAbs have different affinities and can be used to immobilize FcγR.

Fig. 4. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 5. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 6. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 7. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 8. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 9. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 10. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.

Fig. 11. Overlay plot example showing analysis of three different Abs. First capture of His tagged FcγR by antibody with concentration series (25-2000 nM) of anti-His mAb was performed. Followed by a six calibration point concentration series (25-2000 nM) of the FcγR in buffer to study cycle time and curve. Follow-up regeneration.