GE Healthcare

Facilitating IgG FcRn analyses

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Introduction

Therapeutic antibodies are approved for a number of different indications. One important antibody attribute is the Fc Receptor (FcRn) binding properties.

In the present study an assay setup combining biotin capture of FcRn with single-cycle kinetic analysis of the antibody interaction is described.

Further the use of *Dual/ABA inject* for easy analyses of pH dependency is introduced.

The binding mechanism between antibodies and FcRn is heterogeneous due to avidity rendering kinetic analysis of the interaction complex. A simplified statistical approach to SPR analysis, without assuming a particular binding mechanism, is here used for antibody/FcRn analyses.

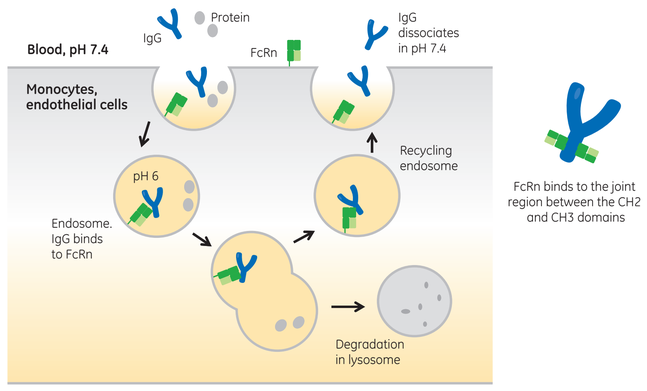


Fig 1. FcRn protects IgG from lysosomal degradation. After pinocytosis IgG binds to FcRn in the endosome and is recirculated out into the blood, thus prolonging the half-life of IgG. Two FcRn molecules are assumed to bind to each IgG Fc with similar affinity (1).

Assay set up using Biotin CAPture Kit

Biotin CAPture Kit:

- Stable, oriented capture of biotinylated FcRn
- Easy to control capture level and change type of FcRn
- Capture kit adds convenience, minimal assay development

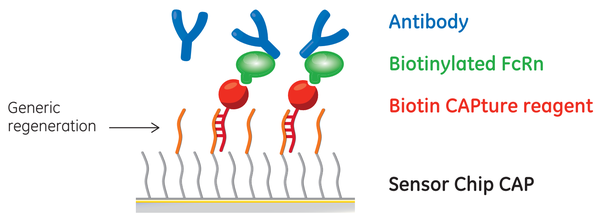


Fig 2. Schematic view of the chip surface of Sensor Chip CAP with immobilized deoxyribo-oligonucleotides. Biotin CAPture Reagent (Streptavidin) is first hybridized to the deoxyribo-oligonucleotides, biotinylated FcRn is captured and analysis of antibody binding performed. The arrow indicates where the regeneration will act on the chip surface.

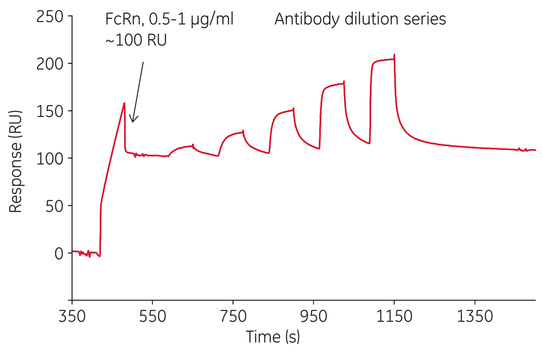


Fig 3. Capture of approx. 100 RU biotinylated FcRn followed by injection of antibody concentration series.

Detailed assay conditions

- CAP reagent, ready-to-use, 5 min injection over both flow cells, obtaining approx. 3000 RU
- Biotinylated FcRn (Immunitrack, Denmark) 0.5-1 µg/ml injected 60 s over flowcell (Fc) 2, to obtain approx. 80-100 RU
- Antibody, single-cycle kinetics 60 s with 300 s dissociation time, over Fc 1 and 2
- Assay buffer: 20 mM phosphate, 150 mM NaCl, 0.05% Surfactant P20, pH 6.0 or 7.4, depending on purpose
- Regeneration: 120 s regeneration solution, Fc 1 and 2. (mix of guanidine hydrochloride and NaOH)

References

1. Abdiche, Y. N. *et al.* 2014, mAbs, 7, 331-338.
2. Neuber, T. *et al.* 2014, mAbs, 6, 928-942.
3. Ober, R.J. *et al.* 2001, Int. Immunol., 13, 1551-9.

Antibody binding profiles for different FcRn species, Biacore T200

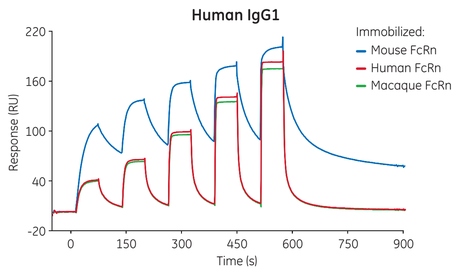


Fig 4. Human IgG1 (25-2000 nM) binding to approx. 100 RU captured FcRn from all species. Binding to mouse FcRn is significantly stronger than to human and macaque FcRn, binding to the latter two being very similar.

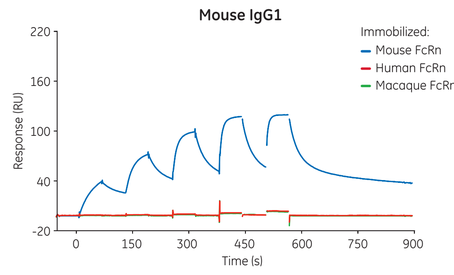


Fig 5. Mouse IgG1 (25-2000 nM) binding only to mouse FcRn (approx. 100 RU captured).

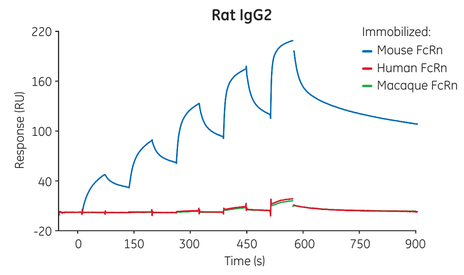


Fig 6. Rat IgG2 (25-2000) binding only to mouse FcRn (approx. 100 RU captured).

→ ***Binding specificities for all antibodies were in agreement with published data (1-3)***

Quantitating similarities using the *Sensorgram Comparison* functionality, Biacore T200

Sensorgram Comparison is an approach to data analysis where the whole, or part of the binding curve is used in the analysis. Sample data is compared to the performance of a standard, here infliximab. The expected variation of the standard data is statistically defined by a comparison window based on upper and lower limit sensorgrams.

A sample that falls inside the comparison window obtains a similarity score of 100%. The further away, i.e. the more deviating a sample is from the comparison window the lower the similarity score.

Results example; hFcRn binding profiles of therapeutic antibodies compared to that of infliximab.

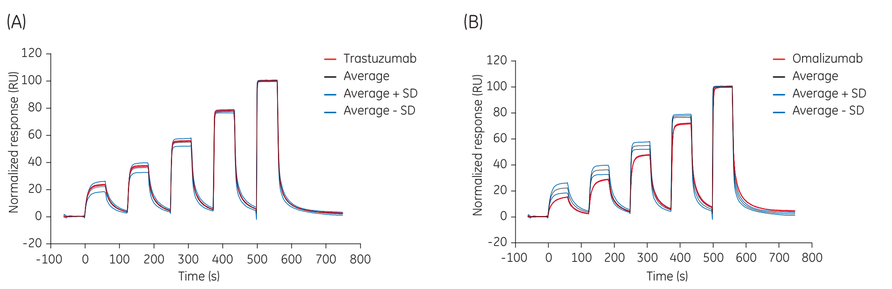
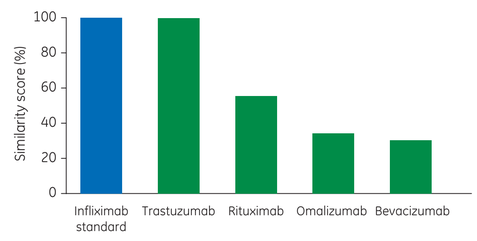


Fig 7. Infliximab was selected as standard (conc. 25–2000 nM), shown as black average curves in (A) and (B) surrounded by 3 SD wide comparison windows, blue. Both association and dissociation were compared in this example. The binding curve of trastuzumab (A, red) fell within the window and trastuzumab received a very high similarity score. In contrast omalizumab (B) has significantly slower on rate and slightly different off rate (red curve) here receiving a similarity score of 34.34%, using these settings.



Ligand	Sample	Similarity score (%)
hFcRn	Infliximab standard	99.92
hFcRn	Trastuzumab	99.72
hFcRn	Rituximab	55.45
hFcRn	Omalizumab	34.34
hFcRn	Bevacizumab	30.42

pH dependent binding analyses facilitated using *Dual inject* or *ABA inject*

Principle using *Dual inject*, Biacore T200

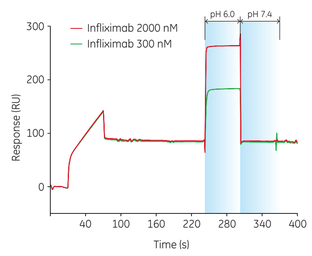


Fig 8. Capture of hFcRn and binding of infliximab (300 and 2000 nM in pH 6.0) followed by immediate dissociation in pH 7.4. Running buffer and FcRn capture buffer was PBS-P+, pH 7.4.

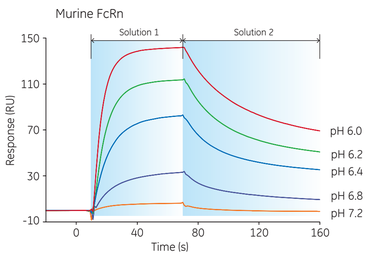


Fig 9. Infliximab (100 nM) interacting with hFcRn, each cycle having the same pH for binding and dissociation.

Principle using *ABA inject*, Biacore 8K

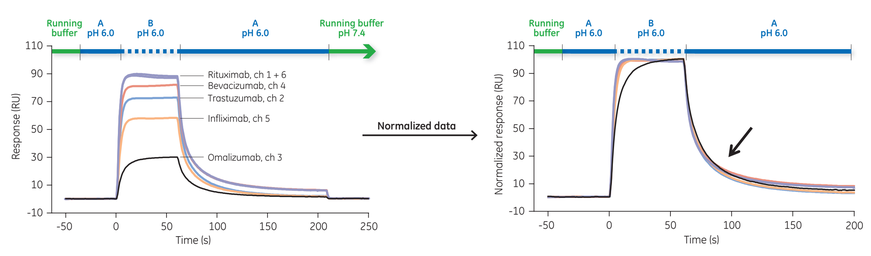


Fig 10. *ABA inject* used to inject 5 antibodies (100 nM in pH 6.0) binding to hFcRn with rituximab replicates in channel 1 and 6. All antibody injections were flanked by buffer, pH 6.0.

Fig 11. Data from Figure 10, normalized between 0 and 100. The antibodies show very similar dissociation at pH 6.0 with omalizumab differing slightly.

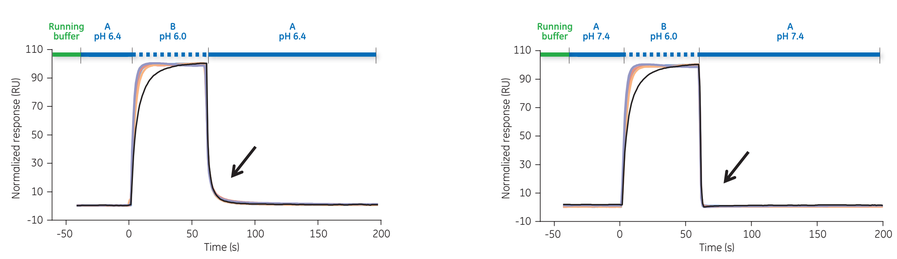


Fig 12. Binding in pH 6.0 with flanking buffer pH 6.4. Faster dissociation for all antibodies compared to data in Figure 11.

Fig 13. Binding in pH 6.0 with pH 7.4 as flanking buffers. Instant dissociation of all antibodies in buffer pH 7.4.

Biacore T100/T200	*Dual inject*
Biacore 2000/3000	*Co-inject*
Biacore S200/8K	*ABA inject*

- Running buffer remains the same, e.g. PBS-P+, pH 7.4
- *Dual inject and ABA inject* solutions are selected freely
- Many antibody analyses may be performed on the same captured FcRn
- Blanks with corresponding pH combinations were included at intervals
- Evaluation: association and/or dissociation rates visually compared

Biacore 8K
- **8 antibodies**
- **3 different pHs**
- **40 min run**

Conclusions

- Minimal assay development using Biotin CAPture Kit for capture of biotinylated FcRn. In combination with single-cycle kinetics both reagents and time are saved.
- *Sensorgram Comparison* quantifies the similarities between a standard and a sample without assuming a binding mechanism, an advantage for heterogeneous interactions like antibody/FcRn.
- *Dual inject* and *ABA inject* facilitates studies of pH dependent binding.
- With a large number of antibodies the time/antibody decreases significantly thanks to flexible use of channels in Biacore 8K.

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