Development of binding assays to measure interactions between Fc regions of therapeutic monoclonal antibodies and Fc receptors using Surface Plasmon resonance.

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Introduction

The therapeutic monoclonal antibody (mAb) market continues to grow as it allows for treaments with higher specificity through direct antigenic targeting. Complex characterization of mAbs is challenging due to their ability to bind to a variety of Fc receptors via their Fc domains, in addition to specific antigen binding via their Fab domain. mAb interactions with Fc receptors result in diverse biological functions associated with each domain. The Fc domain of mAbs interacts with Fc receptors with varying affinities, which can influence biological processes such as Complement Dependent Cytotoxicity (CDC), Antibody-Dependent Cellular Cytotoxicity (ADCC), Antibody Dependent Cellular Phagocytosis (ADCP) and/or serum half-life.

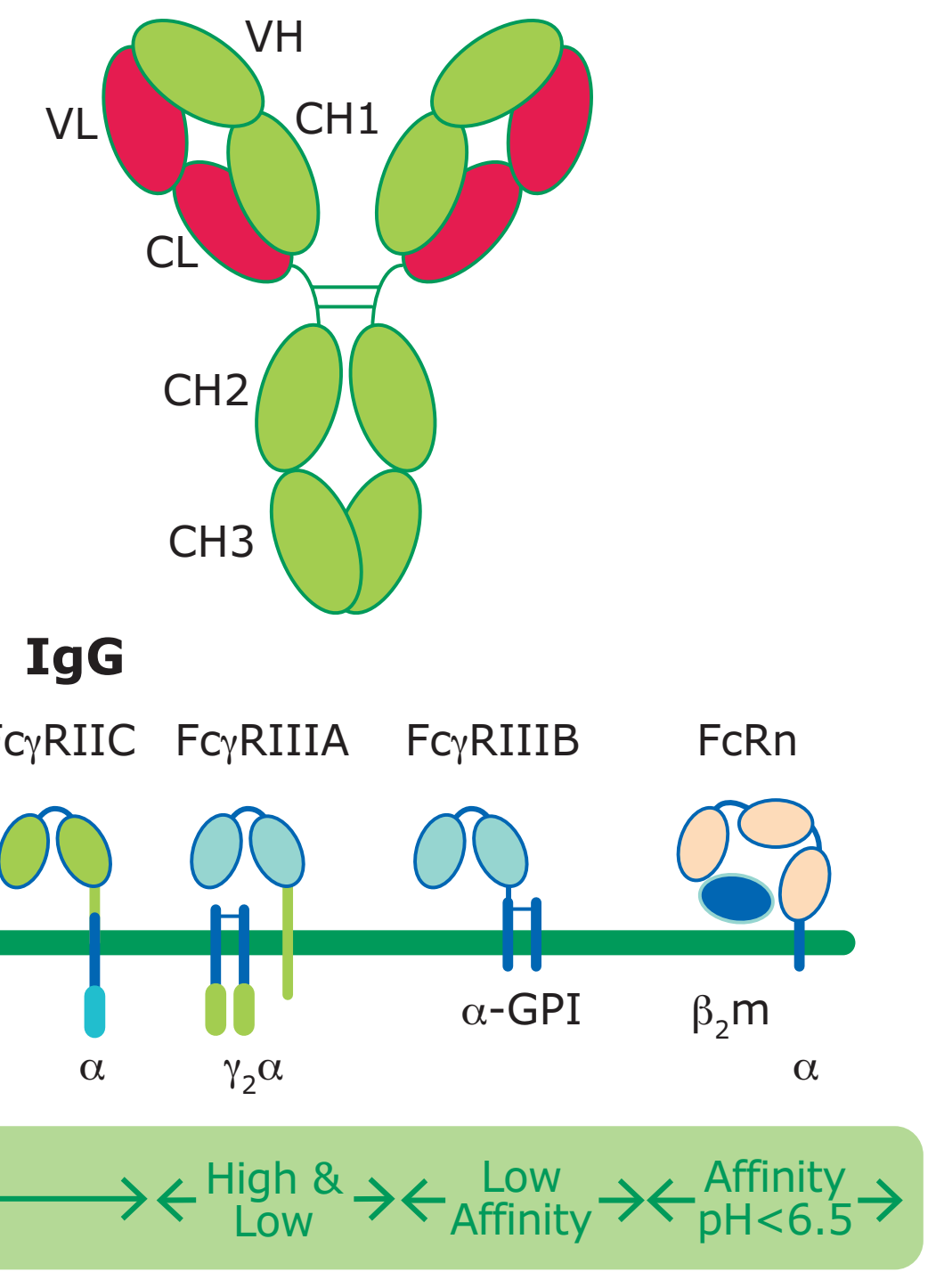
An important characteristic of an antibody is its Fc effector function, and antibodies are now being engineered for optimal binding to Fc receptors expressed on effector cells. Hence it is crucial to evaluate the binding interaction of mAbs with Fc receptors in the early phase of drug development to understand the potential biological activity of the product *in vivo*.

Licensed therapeutic mAbs used:

Adalimumab - Originator molecule that is well known and documented

Cetuximab - Complex originator molecule to showcase our expertise

Commercial purified recombinant Fc receptors



Human IgG receptors: (Pierre Bruhns, Blood 2012;119:5640-5649).
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In the present study, the optimization of binding assays to study the interaction of Fc receptors with therapeutic mAbs is discussed. The assays were developed using the Biacore T200 SPR system to determine the equilibrium dissociation constant, K_D. The high sensitivity of the Biacore instrument provides an excellent platform for FcR-mAb interaction studies.

This study showed distinct binding profiles for the interaction of the two mAbs with Fc receptors, reflecting differences in their structure, glycosylation levels, and serum half-life. These binding assays can be readily modified for optimal performance for specific innovator or biosimilar drug candidates to provide a comprehensive evaluation of the drug candidate's binding characteristics. These assays can be used in all stages of antibody drug development and comparability studies.

Assay Design & Overview

An overview of the binding assay procedure to study the mAb-Fc receptor interaction.

- Running buffer:

Fcγ Receptors ⟶ HBS EP+ pH 7.4.
FcRn Receptors ⟶ HBS EP+ pH 6.0.

- It is known that FcRn binds to IgG in a pH dependent manner. Hence pH 6.0 is used as running buffer.
- Amine coupling used for all receptors.

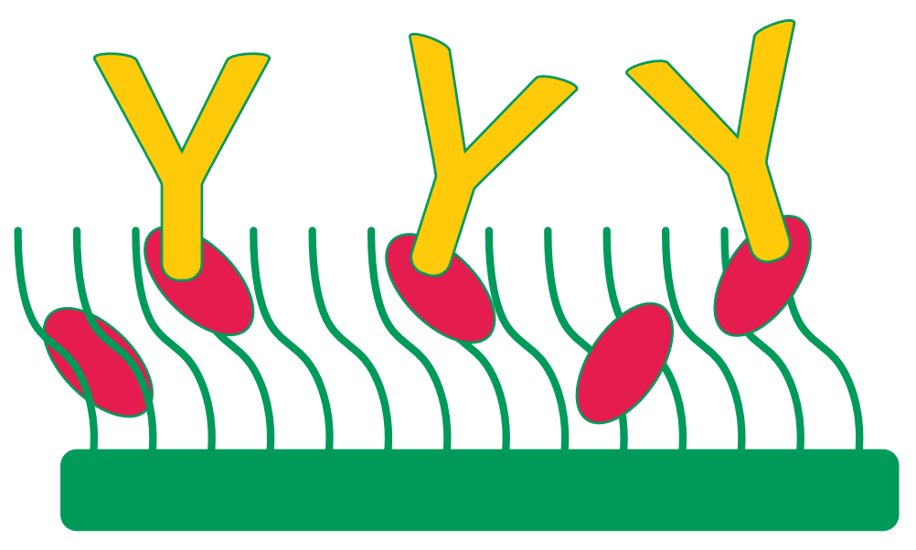
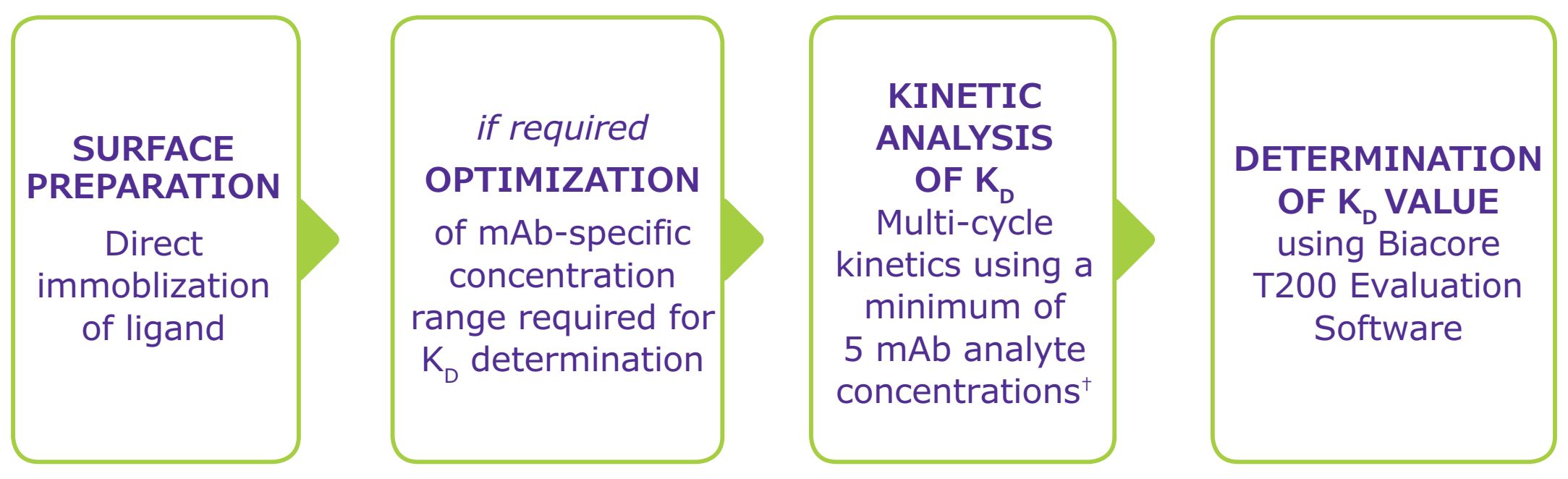


Fig 1. Binding of antibody on SPR.



SURFACE PREPARATION
Direct immoblization of ligand

if required
OPTIMIZATION
of mAb-specific concentration range required for K_D determination

KINETIC ANALYSIS OF K_D
Multi-cycle kinetics using a minimum of 5 mAb analyte concentrations†

DETERMINATION OF K_D VALUE
using Biacore T200 Evaluation Software

Methods

Ligand Immobilization: pH scouting & Amine coupling

pH scouting is used to find the optimal pH of the buffer for immobilizing the ligand, by testing ligand pre-concentration (Figure2, Table1)

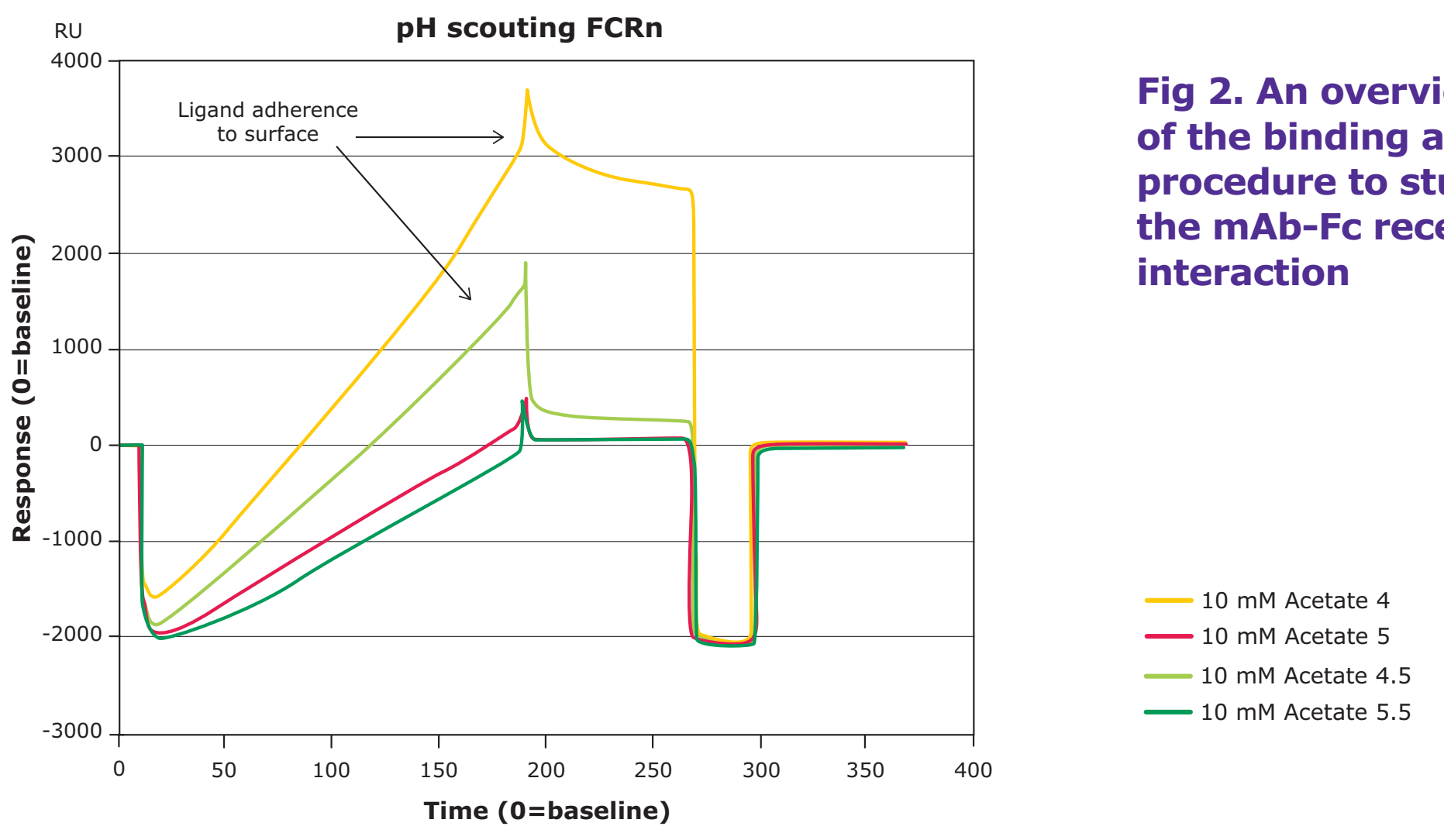


Fig 2. An overview of the binding assay procedure to study the mAb-Fc receptor interaction

Receptor	Immobilization Buffer
FcγRI	10 mM sodium acetate pH 5.5
FcγRIIA	10 mM sodium acetate pH 5.0
FcγRIIB/C	10 mM sodium acetate pH 5.0
FcγRIIIA	10 mM sodium acetate pH 5.0
FcγRIIIB	10 mM sodium acetate pH 5.0
FcRn	10 mM sodium acetate pH 4.5

Table 1. Immobilization buffer used for ligand immobilization

- Immobilization level is generally determined based on the molecular weight of the ligand and analyte, valency of the ligand and the theoretical maximum binding capacity (100RU).
- The ligand level was chosen based on the activity of the receptors.
- Aim for immobilization or custom method used for ligand immobilization.
- Low target ligand level of less than 250 RU was chosen for FcRn.
- A Higher ligand level was chosen for the Fcγ receptors.

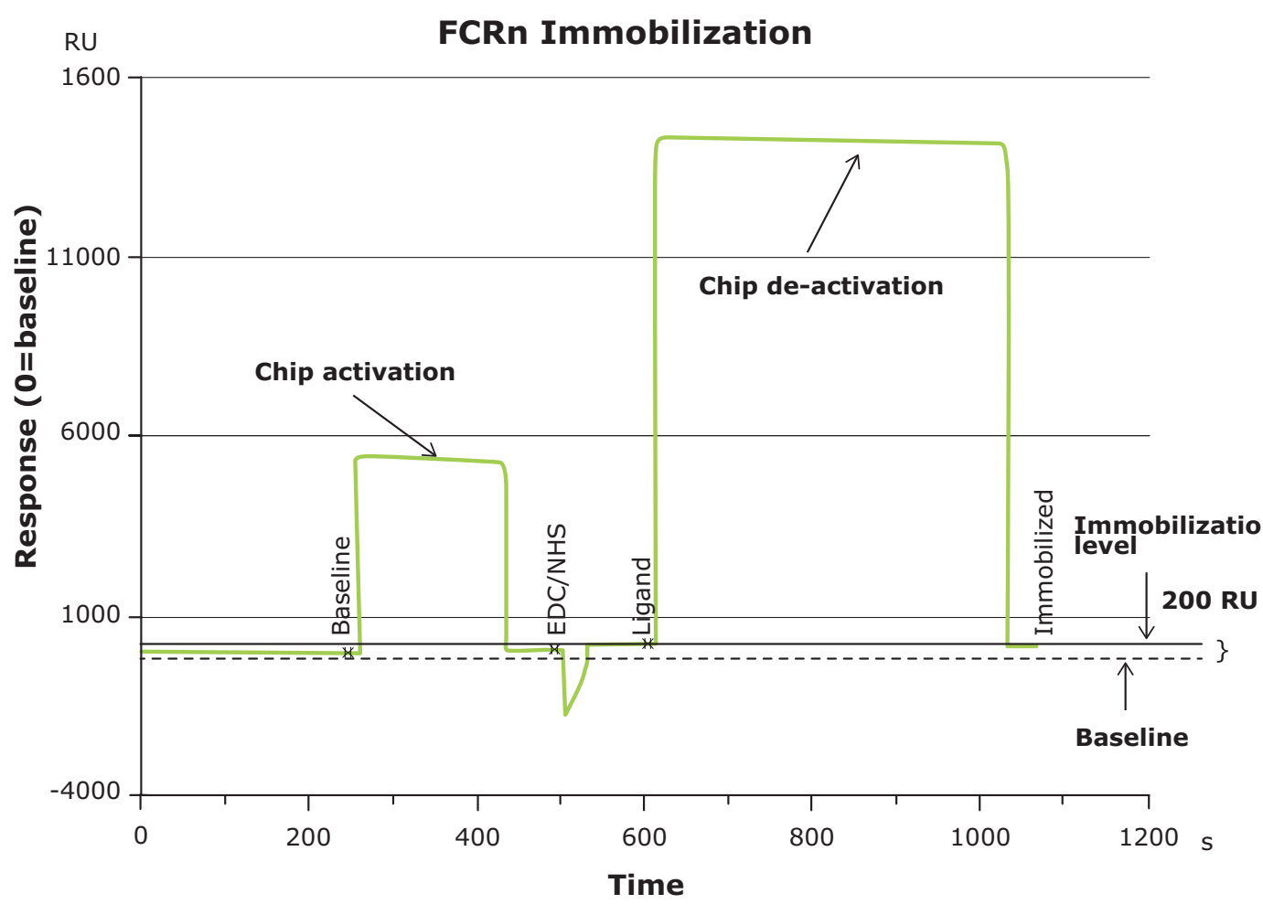


Fig 3. Representative amine coupling result. Sensogram of FcRn immobilization on a CM5 chip using amine coupling.

Analyte Optimization: Regeneration

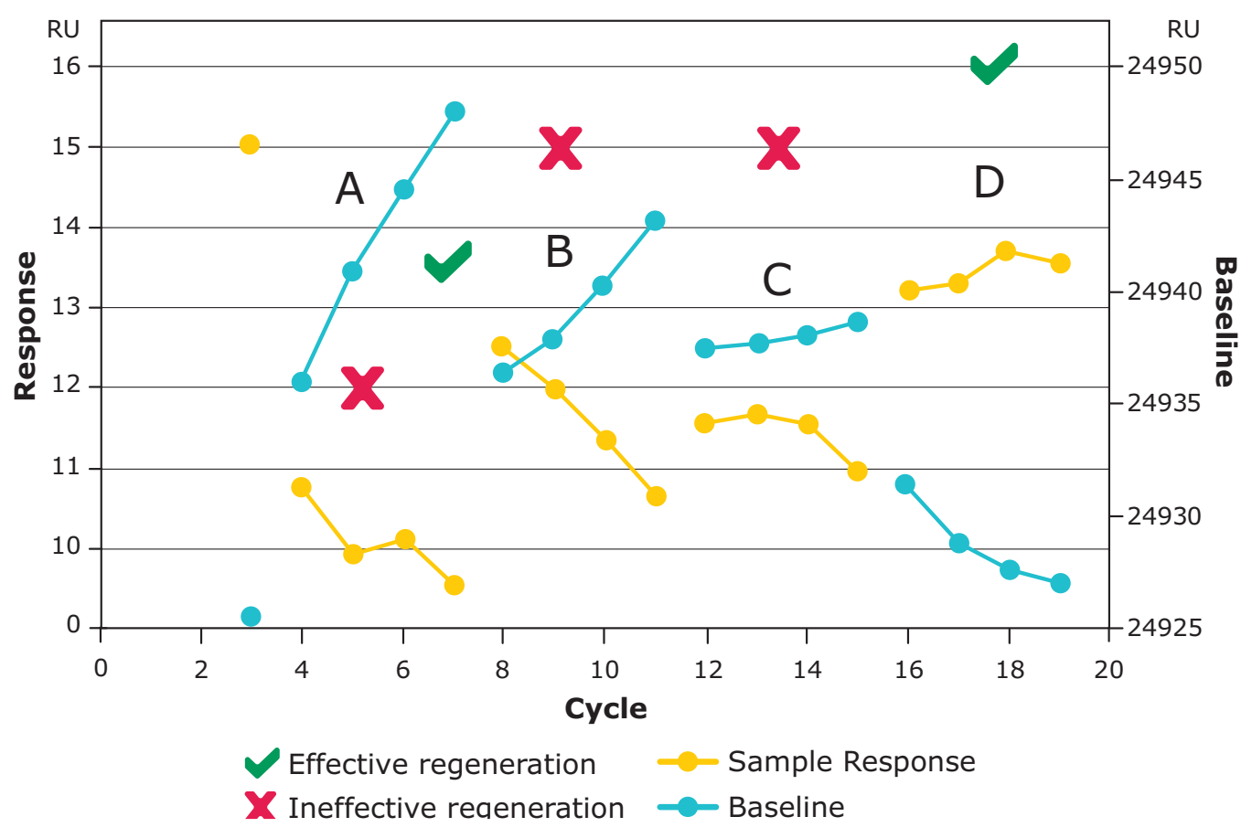


Fig 4. Representative regeneration scouting result for FcRn-Adalimumab (3μM) interaction. The regeneration solutions used were: (a) HBS-EP+ pH 6.0, (b) 0.1M Tris pH 8.0, (c) 0.1M Tris pH 9.0,(d)0.1MTris+ 0.2MNaCl pH 9.0.z

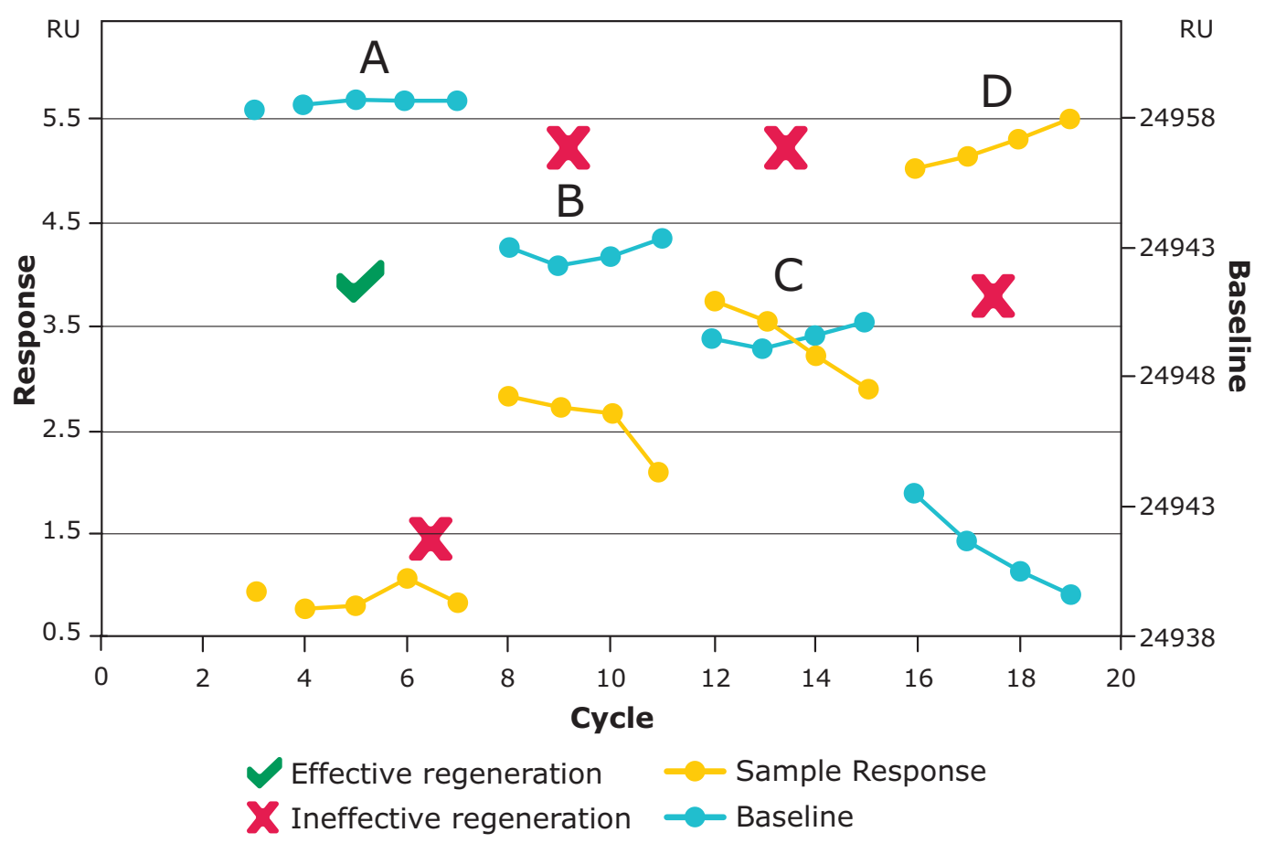


Fig 5. Representative regeneration scouting result for FcRn-Cetuximab (2μM) interaction. The regeneration solution used were: (a) HBS-EP+ pH 6.0, (b)0.1M Tris pH 8.0, (c) 0.1M Tris pH 9.0, (d) 0.1MTris+ 0.2MNaCl pH 9.0.

Receptor	Cetuximab	Adalimumab
FcγRI	10mM NaOH	10mM NaOH
FcγRIIA	HBS-EP+ pH 7.4	1 M Tris 8.0
FcγRIIB/C	HBS-EP+ pH 7.4	1 M Tris 8.0
FcγRIIIA	HBS-EP+ pH 7.4	10mM NaOH
FcγRIIIB	HBS-EP+ pH 7.4	HBS-EP+ pH 7.4
FcRn	HBS-EP+ pH 6.0	0.1 M Tris + 0.2 M NaCl pH 9.0

Table 2. Regeneration conditions for various receptors and the mAbs

Analyte Optimization: Surface performance

- Surface performance test using wizard or manual injection is used to access the robustness of the surface and quality of regeneration.
- Association and Dissociation times were also determined during surface performance.
- Regeneration Scouting: Appropriate regeneration condition is required to remove the mAb from the surface when using it for various analyte concentration cycles.
- Regeneration scouting wizard or manual injection was used to determine the regeneration conditions for the sensor surface

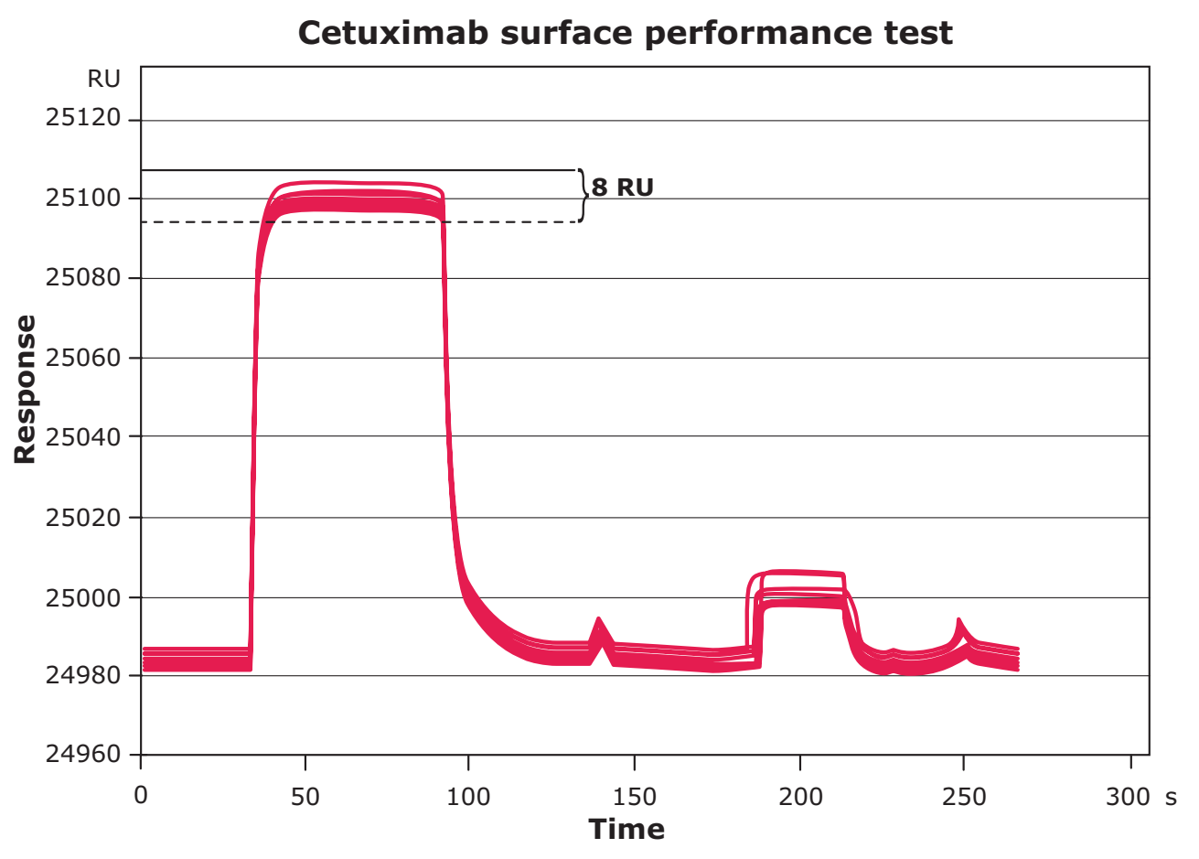


Fig 6. Representative surface performance result. Binding response of 2700 nM Cetuximab to FcRn from cycle 1 to cycle 40 (8 RU change).

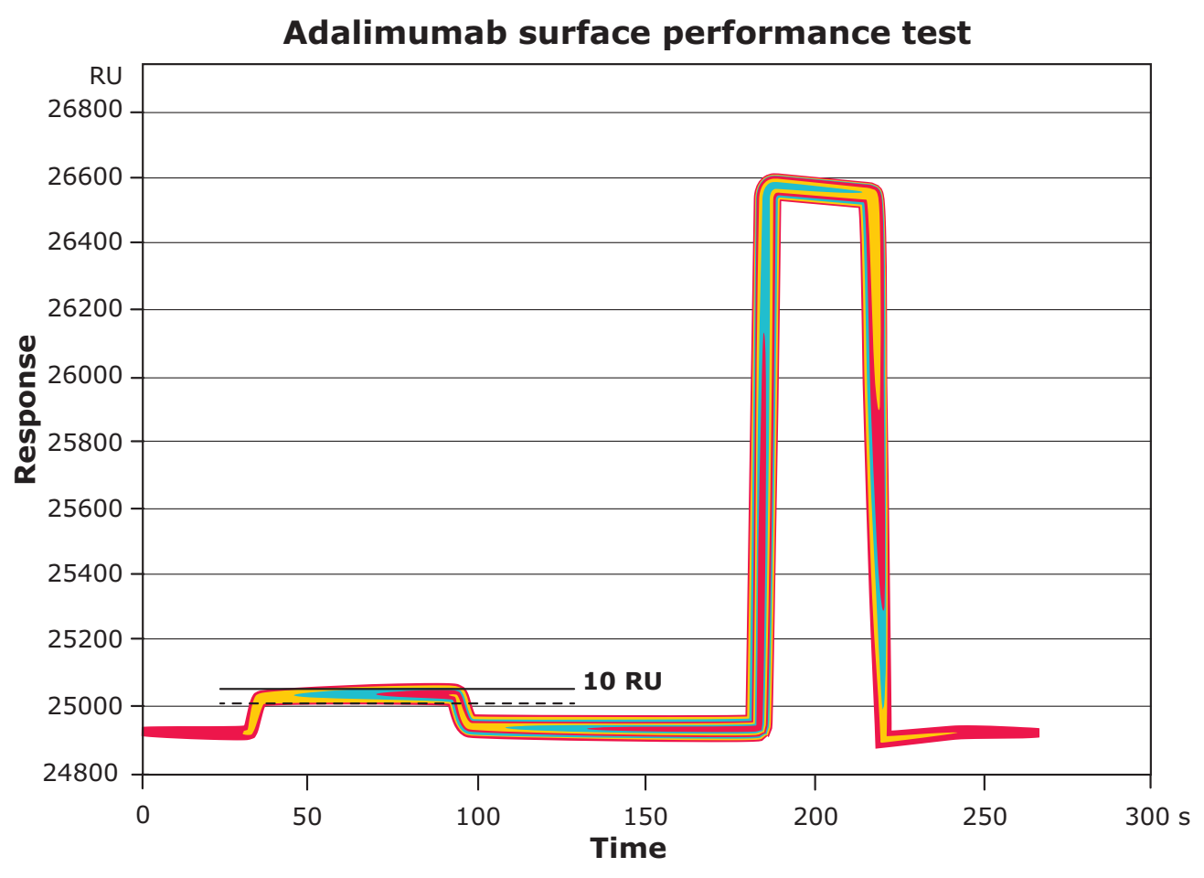


Fig 7. Representative surface performance result. Binding response of 900 nM Adalimumab to FcRn from cycle 1 to cycle 40 (10 RU change).

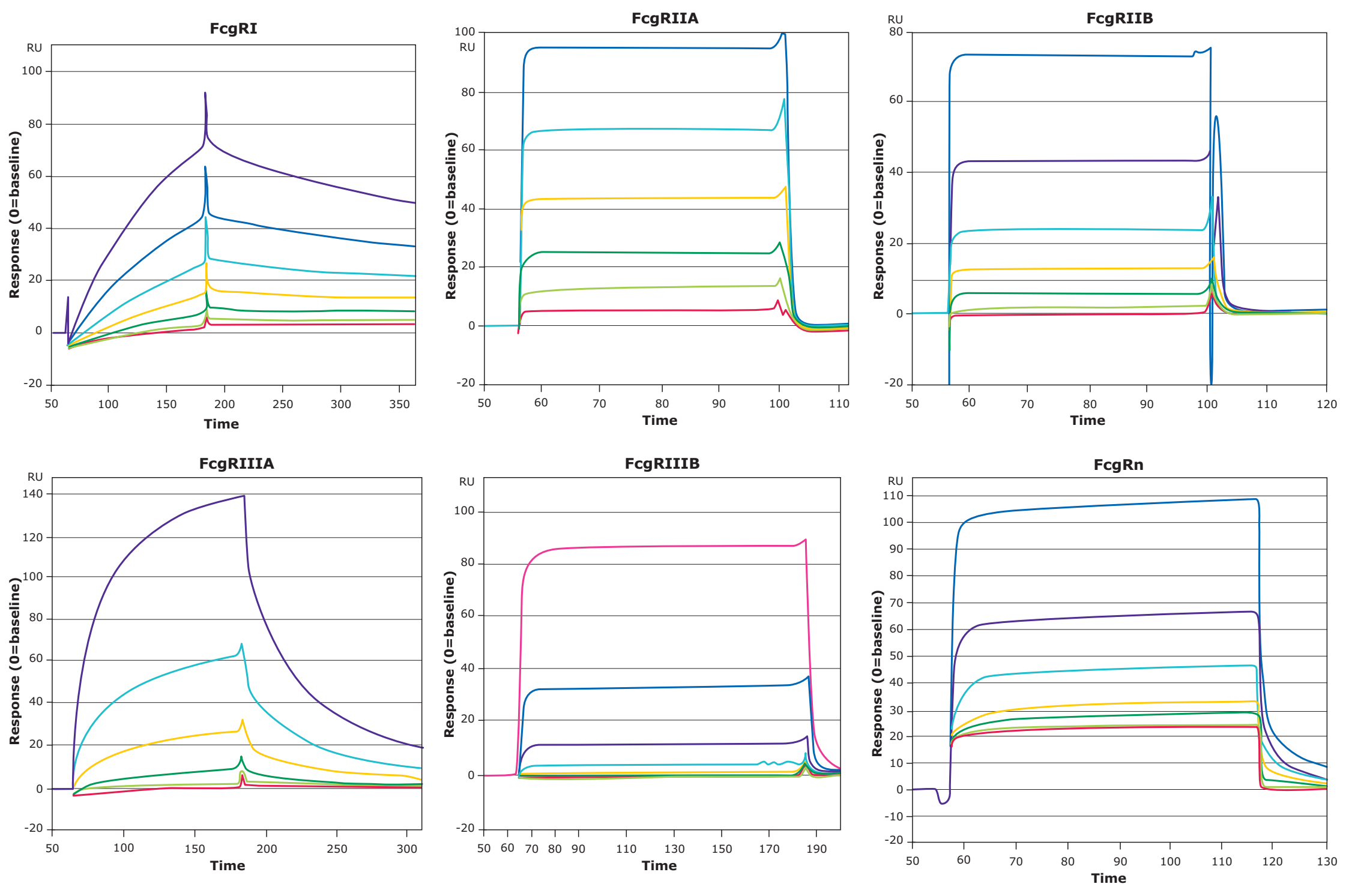


Fig 8. Representative mAb analyte response from the interaction with Fc receptors

Receptor	Adalimumab (nM)	Cetuximab (nM)
FcγRI	0.55-35	1-111
FcγRIIA	125-8000	125-8000
FcγRIIB/C	125-8000	125-8000
FcγRIIIA	1-250	13-1614
FcγRIIIB	1-11261	13-13157
FcRn	1.23 -900	3.7 -2700

Table 3. Optimized mAb Concentration range used for the binding assays

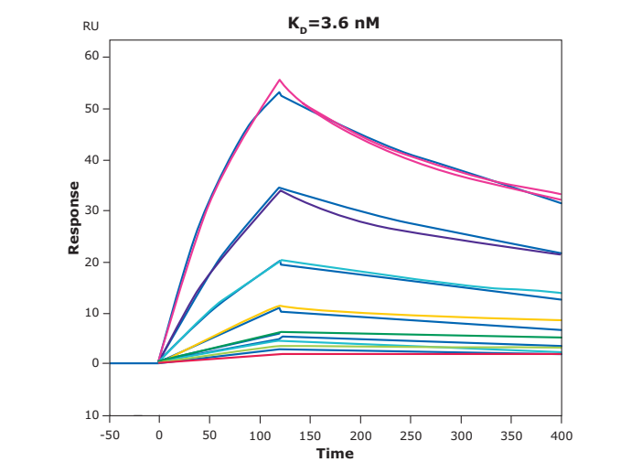
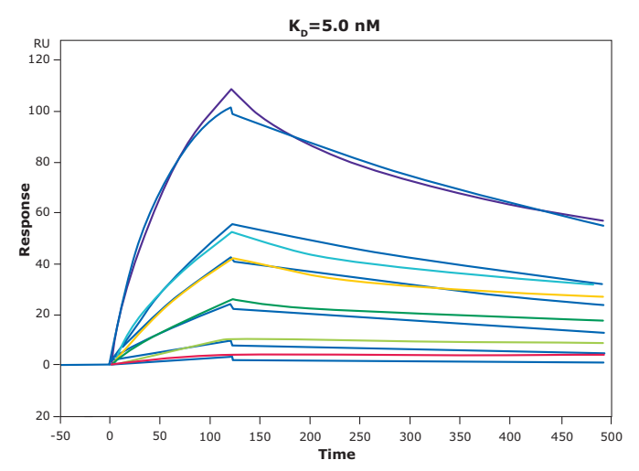
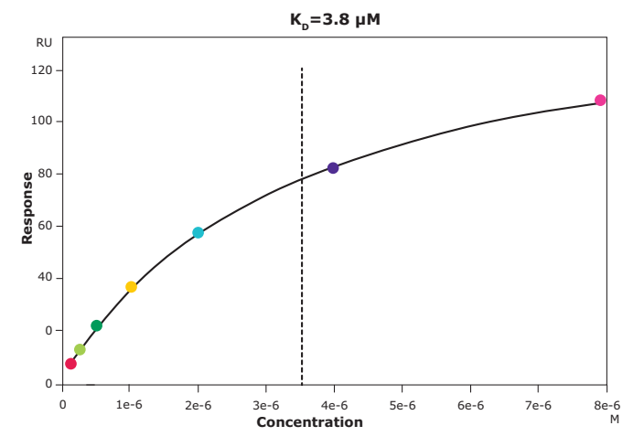
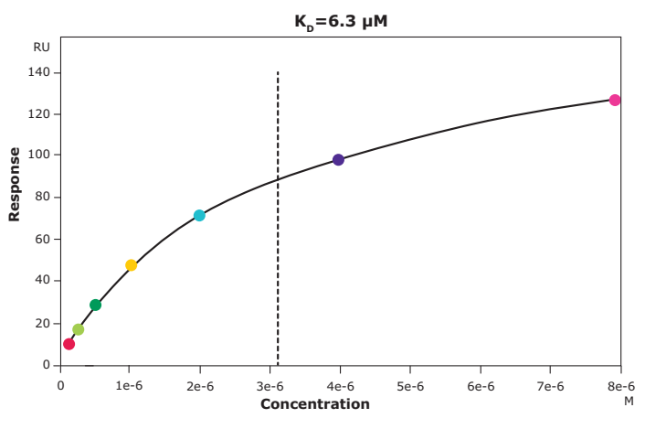
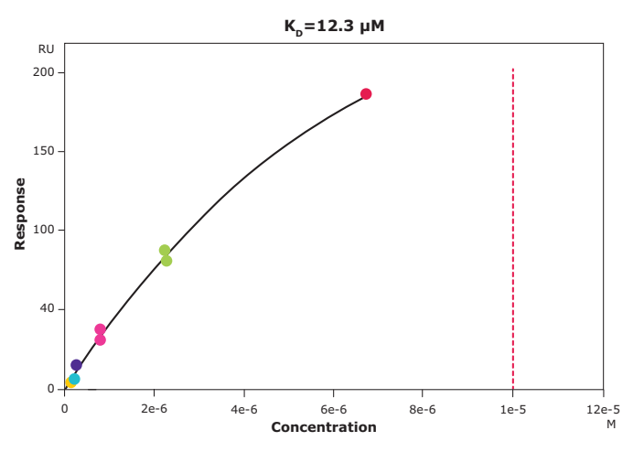
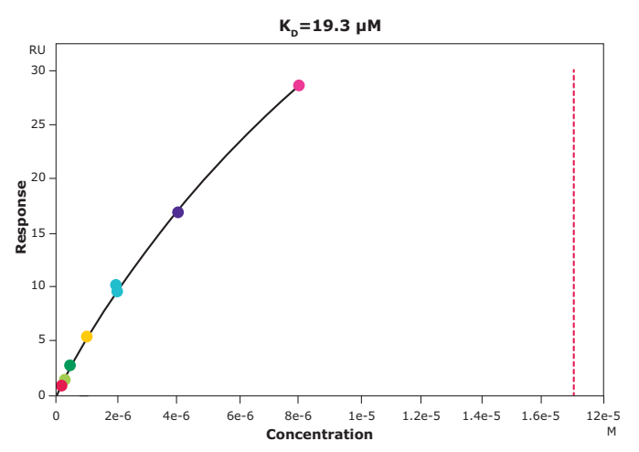
Data Analysis

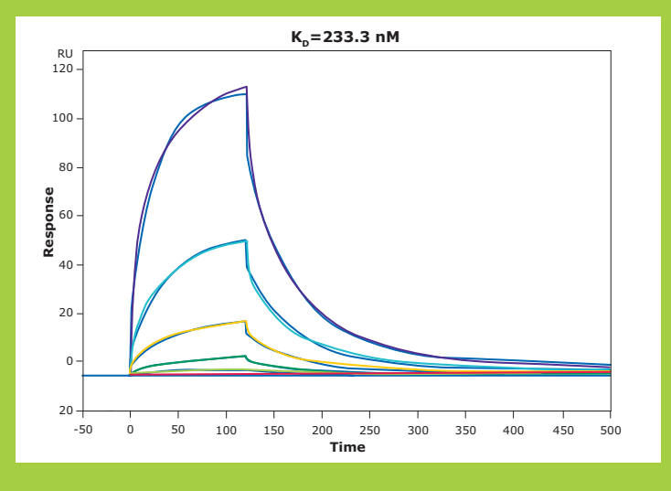
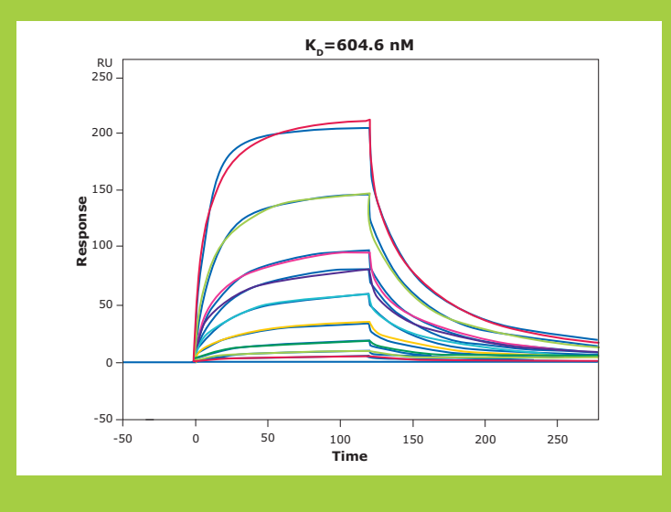
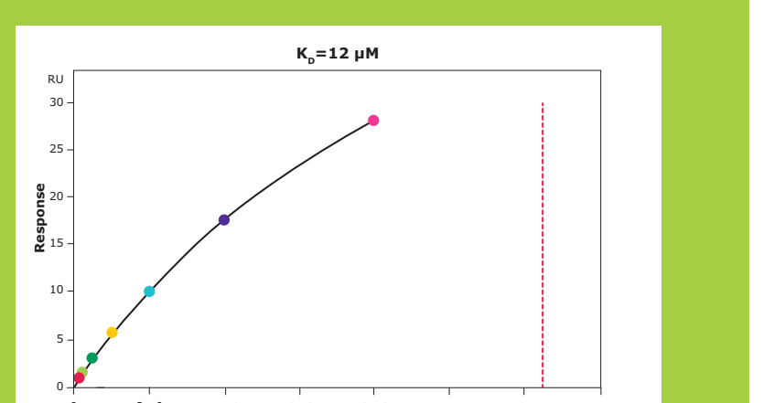
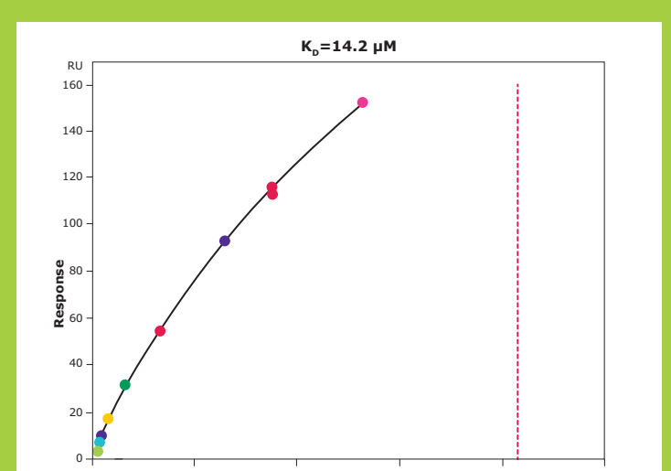
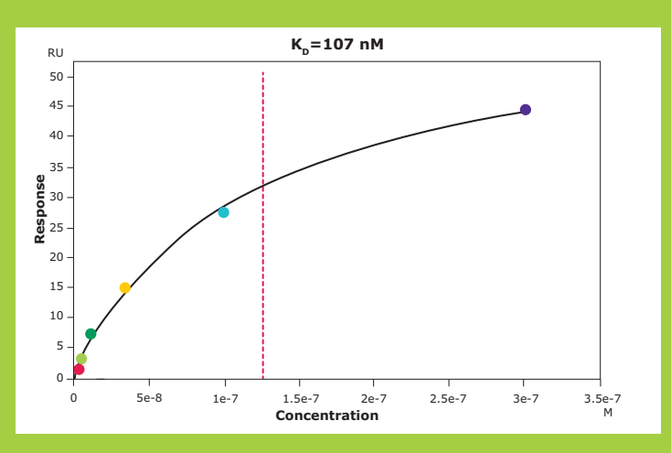
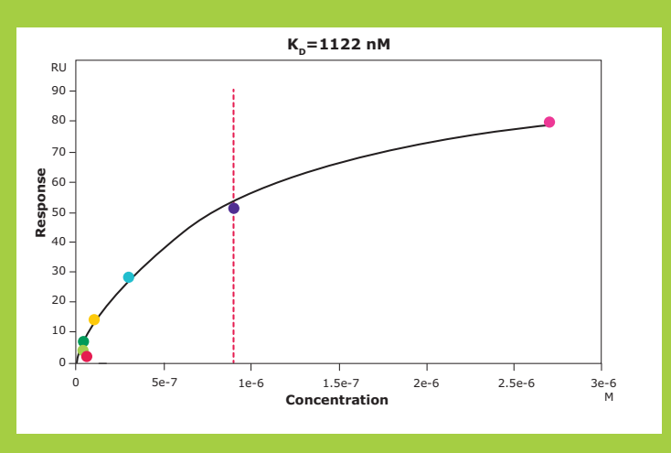
- Appropriate kinetic models were used for the K_D determination based on the Fc receptor mAb interaction
  - 1:1 binding model: FcγRI, FcγRIIIA
  - Steady state affinity model: FcγRIIIB, FcγRIIA, FcγRIIB, FcRn
- % Chi^2 of Rmax value < 20% of the Rmax for the steady state analysis.
- % Chi^2 of Rmax value < 10% of the Rmax for the kinetic analysis.
- tc values should be greater than 10^8.

Results

- For all the receptors, Cetuximab has lower binding affinity compared to Adalimumab
- Adalimumab relative Fc binding affinities (K_D) : FcγRI > FcRn > FcγRIIIA > FcγRIIA > FcγRIIIB > FcγRIIB.
- Cetuximab relative Fc binding affinities (K_D) :FcγRI > FcγRIIIA(V) > FcRn > FcγRIIA > FcγRIIIB > FcγRIIB

Table 4. Data fitting for the mAb-Fc interaction with intermediate precision in K_D , % CV, and number of experiments.

Receptor	Adalimumab (nM) Intermediate precision K_D (nM)	Cetuximab (nM) Intermediate precisionK_D (nM)
FcγRI	 K_D=3.6 nM	 K_D=5.0 nM
FcγRIIA	 K_D=3.8 μM	 K_D=6.3 μM
FcγRIIB/C	 K_D=12.3 μM	 K_D=19.3 μM

Receptor	Adalimumab (nM) Intermediate precision K_D (nM)	Cetuximab (nM) Intermediate precisionK_D (nM)
FcγRIIIA	 K_D=233.3 nM	 K_D=604.6 nM
FcγRIIIB	 K_D=12 μM	 K_D=14.2 μM
FcRn	 K_D=107 nM	 K_D=1122 nM

Summary

- Binding assays were developed to perform kinetic, real-time analysis of the binding interaction between Fc receptors and the Fc region of therapeutic monoclonal antibodies (mAbs), using SPR technology.
- The assays were developed using a Biacore T200 instrument, which uses direct immobilization of recombinant Fc receptors, followed by kinetic binding analysis in order to generate an equilibrium dissociation constant (K_D) value.
- As expected, the analyzed mAbs exhibited highest affinity to FcγRI and the inhibitory receptor FcγRIIB/C has a lower affinity compared to all the other Fc receptors.
- Adalimumab had higher affinity to FcRn than Cetuximab, as reflected in the half-life of the antibodies which is on average 15 and 5.5 days for Adalimumab and Cetuximab, respectively.

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