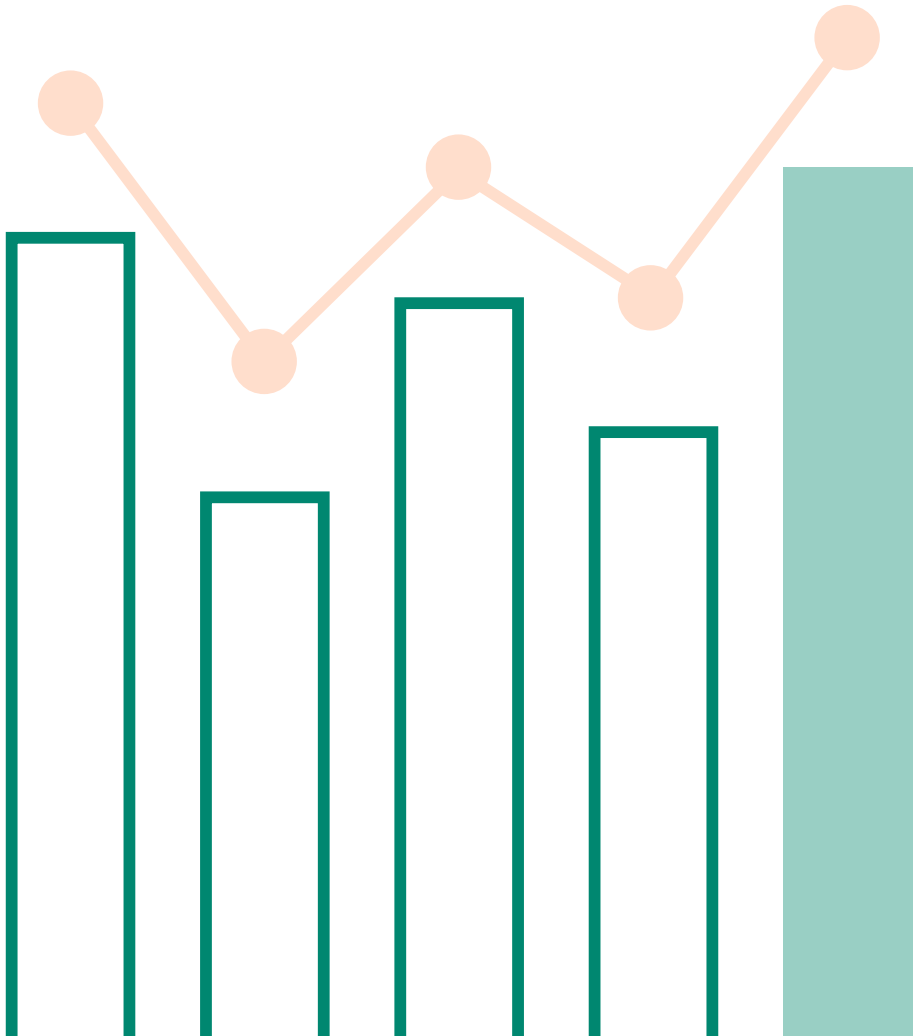
cytiva

Biacore™ surface plasmon resonance systems in late-stage development and quality control of biotherapeutic drugs

Biotherapeutic drugs including antibodies, cytokines, and hormones are used for the treatment of a variety of diseases including arthritis, cancer, and diabetes. Compared to traditional small-molecule drugs, their structures are complex, and they can assert their effects by binding with high specificity to more than one target molecule. During development and quality control, you may use a range of analytical technologies to characterize biotherapeutic drugs in terms of their structural integrity and activity. In this white paper, we review the use of Biacore™ surface plasmon resonance (SPR) systems for active concentration measurements, target binding, potency (EC50), and fragment crystallizable (Fc) receptor analysis and describe how you can use these assays for assessment of drug potency and stability.



Introduction

The life cycle of a biotherapeutic product typically spans over 30 years (yr) as illustrated in Figure 1. Late-stage development includes process development, formulation, and clinical trials, and can extend over 3 to 5 yr depending on the duration of clinical trials. Initial process development within the late-stage development is rapid, approximately 1 yr. The process is refined in parallel with the first clinical trials. A successfully developed product can remain in manufacturing and quality control for over 20 yr with additional indications added over time. Successful biotherapeutics such as Rituxan, Herceptin, Enbrel, Remicade, Humira, Avastin, and Lantus, which were first approved between 1997 and 2004, all serve as examples of these timelines.

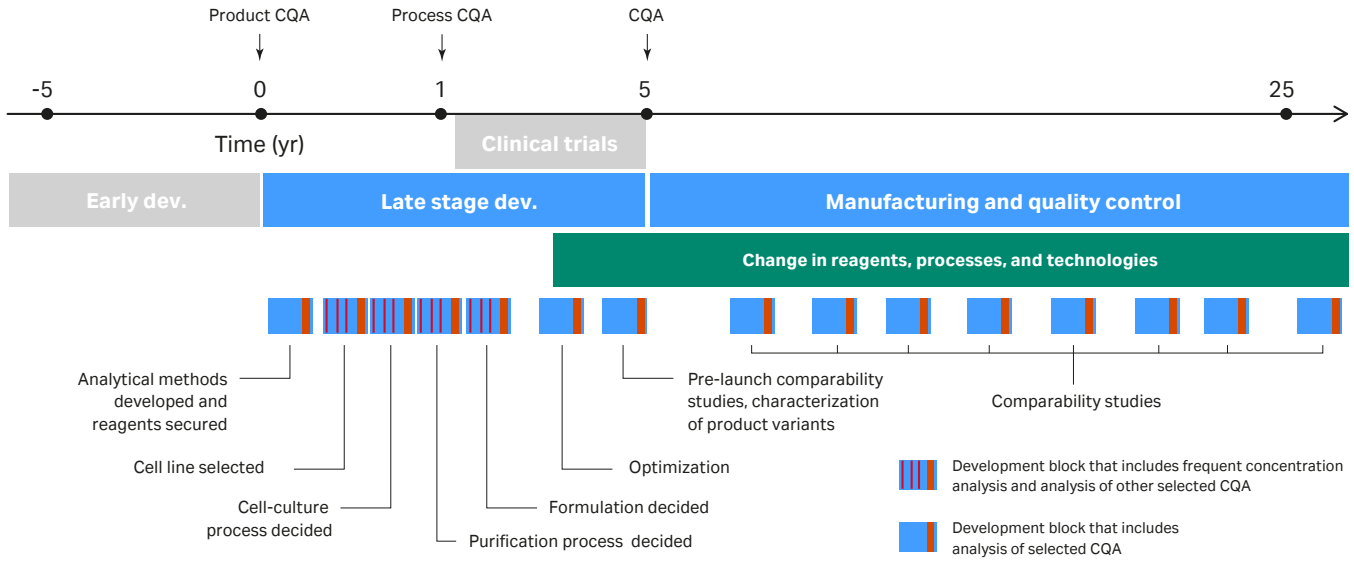


Fig 1. Events in the life cycle of a biotherapeutic drug. Each development block and comparability study include analysis for CQA.

Biotherapeutics including monoclonal antibodies (mAb) are the key part of many emerging medicines. Other modalities like bispecific and multispecific antibodies, antibody-drug conjugates ADCs, mRNA- based drugs, and targeted protein degraders have also emerged in recent years. The number of FDA-approved antibodies has increased rapidly in recent years. In 2021, the FDA approved the 100th monoclonal antibody product (1).

In the early phases of development, your focus is on the drug substance and the assumed mechanism of action (2). Early biotherapeutic development is typically target-based (3). For a therapeutic antibody, the mechanism of action includes target binding but can also include Fc receptors and complement binding. For cytokines and hormones, receptor binding is essential. Biacore assays like screening and epitope binning are widely used in early-stage development (4).

The antibody development workflow (Fig 2A, 2B) has evolved, and early development is no longer focused entirely on potency and functional aspects such as specificity, affinity, and kinetics for their molecular targets. While these factors are crucial, developability aspects play an increasingly important role in reducing the risk of a later failure of the development program. Developability aspects include studies on: (i) the impact of post-translational modifications on stability and conformation, (ii) aggregation and fragmentation tendencies, (iii) solubility and solution stability, (iv) biological factors such as immunogenicity and pharmacokinetic properties. Developability studies try to answer questions such as: Can the antibody be manufactured, is it safe, and will it have acceptable bioavailability and efficacy?

As your lead candidate enters late-stage development, several “critical quality attributes” (CQA) (Fig 2C), that is, properties that ensure clinical safety and efficacy, including data on how it interacts with target proteins, are established. You can identify important process-related CQA such as protein integrity, homogeneity, presence of host cell proteins, host cell DNA, and substances released from process or package material using risk assessments based on previous experience and knowledge and by control procedures. For each step in the process, you can identify critical process parameters (CPP) that can affect CQA (5), and a control strategy is defined.

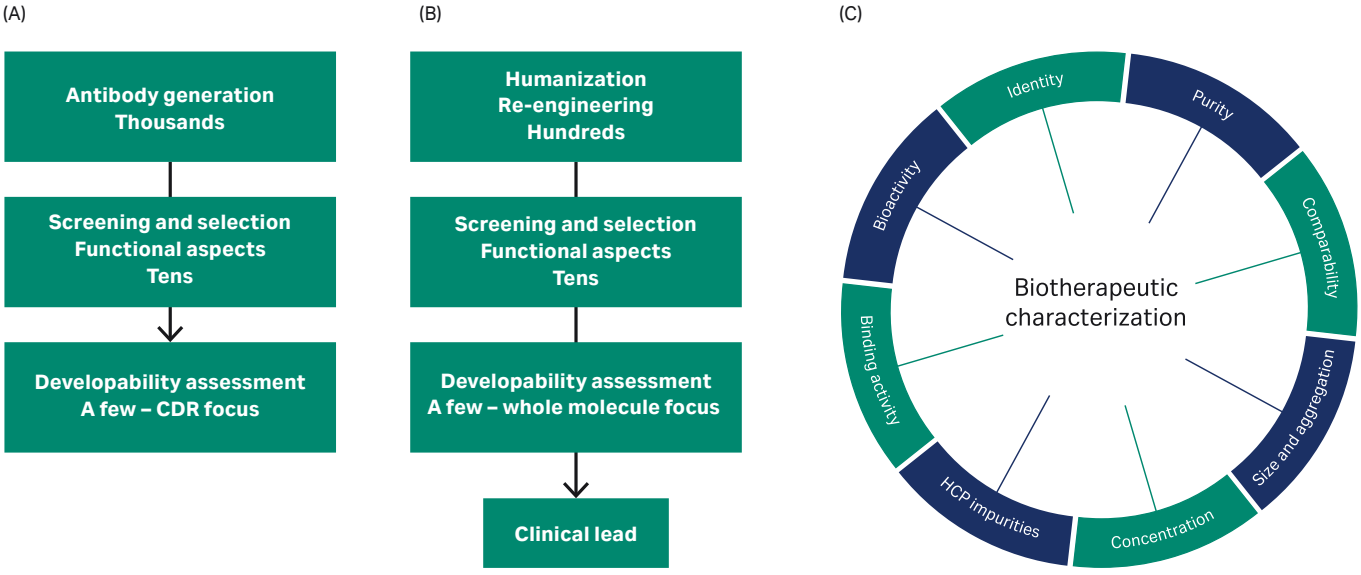


Fig 2. The antibody development workflow: (A) first selection round with focus on antigen binding, (B) re-engineering and selection of clinical lead, and (C) developing the methods for CQA for the antibody characterizations. You can use Biacore SPR systems for binding activity, affinity, kinetics, concentration measurements, potency, and comparability studies.

This white paper focuses on Biacore assays that are useful to you in late-stage development, manufacturing, and quality control of biotherapeutics. The assays discussed here are typically performed in analytical labs, which receive samples from process development, formulations, or production groups. In the analytical lab, analysis is focused on the control of cell culture (6) and purification (7) procedures, for example, cell culture titer analysis, post-translational modification assessment, impurity testing, concentration and potency assay, and stability studies. With CPP identified and a control strategy in place, the CQA should be secured, and verification should be straightforward. To support this process, requirements on test procedures and acceptance criteria have been described in regulatory guidelines (8) for biotechnological/biological products.

Development starts with the setup and validation (9) of analytical methods and with securing reagents (10, 11) for the analytical program. You may use a broad range (50 to 60 variants) of different analytical technologies for CQA analysis (12). Mass spectrometry (MS) can be used to establish the identity of the drug (primary sequence) and for the detection of size distribution profiles linked to post-translational modifications such as glycosylation (13). You can also use MS in combination with high-performance liquid chromatography (HPLC) and other chromatographic methods to detect and localize amino acid modifications (14). Chromatography techniques are broadly used for the detection and isolation of charge and size variants (15). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting demonstrate protein integrity, and in the case of antibodies, the presence of HHL-, HL- or L chains can easily be detected (16). Enzyme-linked immunosorbent assay (ELISA) and SPR are commonly used for in vitro confirmation of biological activity by measuring interactions with antigens, receptors, Fc receptors, or other binding proteins (12, 14).

Process development for chemical manufacturing and control add brackets (CMC) (17) includes the selection of the cell line for protein expression, development of the cell culture process, and purification of the drug substance, while formulation includes selection of excipients for the final drug product. During process development, the goals are to obtain high product yield and good process economy to ensure that properties related to initial CQA are maintained and to secure that the process itself is well controlled with low impurity levels. Formulations ensure that your final product has proper stability, administration properties, and pharmacokinetic profile. Throughout all steps of late-stage development, initial CQA is monitored and possible new CQA is evaluated. Before transfer to manufacturing, the complete list of CQA is established along with methods for their control. Manufacturing and quality control (QC) ensures a supply of products with consistent quality to the market. CQA is determined using knowledge at a given time and might later have to be revised based on clinical data that accumulate over time.

Manufacturing timelines extending over decades constitute both challenges and opportunities. The challenge is to maintain a high and consistent product quality while manufacturing analytical technologies evolve and materials and reagents become obsolete and must be replaced. The opportunity lies in improvements that simplify your processes and make them more reliable and/or more economic. The demonstration of comparability (18) does not necessarily mean that the quality attributes of the pre-change and post-change products are identical. However, based on results and existing product knowledge, it should be possible to predict that any differences in quality attributes have no adverse impact upon the safety or efficacy of the drug product. Manufacturing changes can be frequent (19) and approval times for changed products can be lengthy.

While comparability studies put pressure on using modern analytical technologies, an even stronger push for extended and more detailed fingerprint analysis comes with the development of biosimilars (20, 21, 22, 23). Biosimilars are generic products that are physically “identical” to an already approved drug and with the same safety and efficacy profile as that of the reference product. A biosimilar should mimic the reference product in terms of properties but the processes and technologies used for manufacturing and control can be different from that used for the reference product. The results from extensive analytical comparisons with the reference product may serve as an indication of the extent of clinical trials that have to be performed.

Biacore SPR assays are based on ligand binding

Ligand-binding assays are key for the characterization of biotherapeutic medicines and are extensively being used with SPR and ELISA. Our Biacore systems and ligand binding assays are based on SPR analysis and have been used for antibody characterization for more than 30 yr.

The readout from a Biacore system is related to molecular mass and you can detect any binding event without the use of labels. The readout is continuous, which allows quality control of the entire binding event. This provides opportunities for data analysis based on binding responses obtained at one or several specific time points (report point analysis) or by comparing and even fitting entire binding curves for the determination of kinetic and affinity parameters. Biacore ligand-binding assays are direct-binding assays (24) focusing on relevant interactions (25). They provide information on binding activities and can detect strong and very weak interactions. This contrasts with ELISA, which is an end-point assay where detection is based on the use of a labeled secondary reagent, where the readout is obtained at a less well-defined time point, and where weak interactions might go undetected.

ELISA and Biacore SPR assays can be used in direct and competitive formats in a variety of situations where binding data is required to confirm and quantitate the presence of an analyte or for characterization and confirmation of biological activity. Characterization of binding events is essential for confirmation of CQA as outlined in Figure 2. Assay setups are often similar between ELISA and Biacore systems although there is no guarantee that assay reagents are interchangeable from one platform to the other.

Biacore SPR assays allow you to plot data from several samples in overlay plots as illustrated in Figure 3. This effective data display makes data from Biacore SPR systems relatively easy to interpret. Biacore SPR data is often more precise than ELISA (26, 27), presumably as Biacore SPR assays involve fewer steps and are under strict time and temperature control.

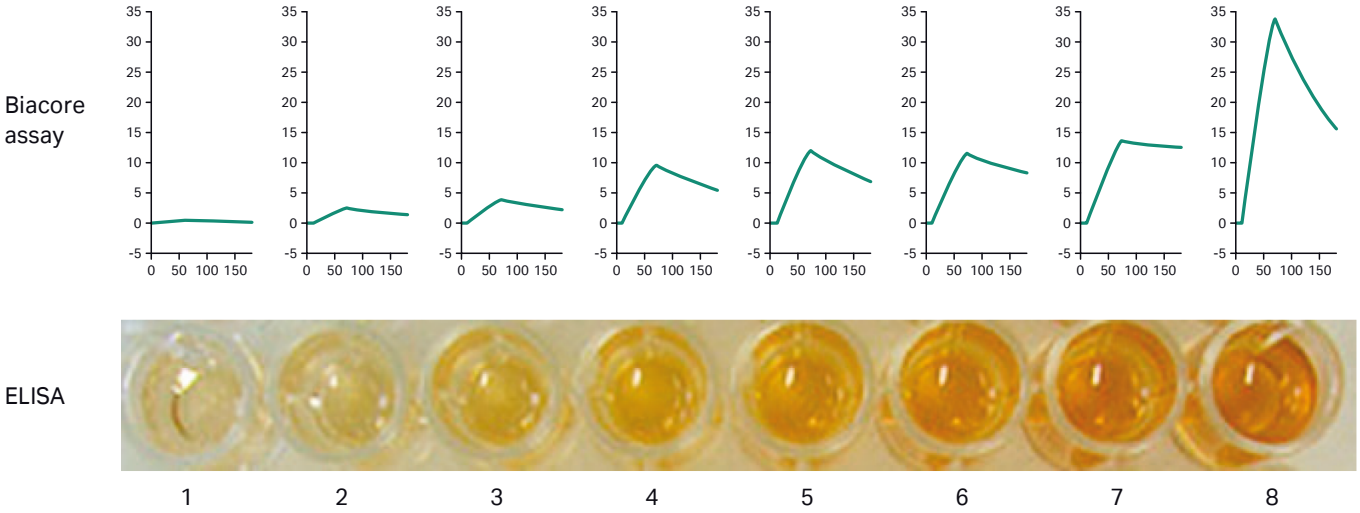


Fig 3. Hypothetical ELISA and Biacore SPR assay responses. Increasing ELISA responses in wells 1 to 8 with Biacore binding curves displayed above the wells. The higher binding level initially observed in the Biacore ligand biding assay for the sample in well 8 and the higher stability of sample 7, are examples of information that you cannot deduce from ELISA experiments alone.

Biacore SPR systems measure titer and active concentration with high precision

Concentration analysis is vital in several process development steps: to determine the yield in cell line development (CLD), cell cultures over time; for identification of suitable chromatography conditions; for monitoring of final purification processes; and in formulation studies.

Figure 4 illustrates the dynamic range and precision in concentration analyses using Biacore systems. The assay readout is from a report point at the end of sample injection. In these examples, an antihuman Fc antibody is immobilized on the sensor surface. In Figure 4A, omalizumab at concentrations from 2 ng/mL to 1 mg/mL was injected for a 3 min period. The insert shows the response for the lowest (2 to 8 ng/mL) concentrations. This assay can be fine-tuned and, depending on the concentration range of interest, the analysis time can be adjusted. In Figure 4B, the sample injection time was reduced to 20 s and the concentration range of interest was from 0.5 to 50 µg/mL. The figure shows data obtained with repeated injections of standards, controls, and one selected sample over 1000 analytical cycles obtained on the same surface. Noticeably, standard and control samples were stable over time and the coefficient of variation for the repeat sample was 1.3%. Clearly, Biacore SPR assays are sensitive, have the potential for a wide dynamic range, can be very rapid, and, based on the Biacore system design, can be multiplexed with the possibility to run several assays in parallel. Validation of a Biacore SPR assay for antibody analysis considering ligand coupling and regeneration procedures, buffer conditions, sensor chip storage, inter-assay variability, the limit of detection, and quantitation is described in reference 24, which additionally describes the use of a Biacore SPR assay in a GMP environment.

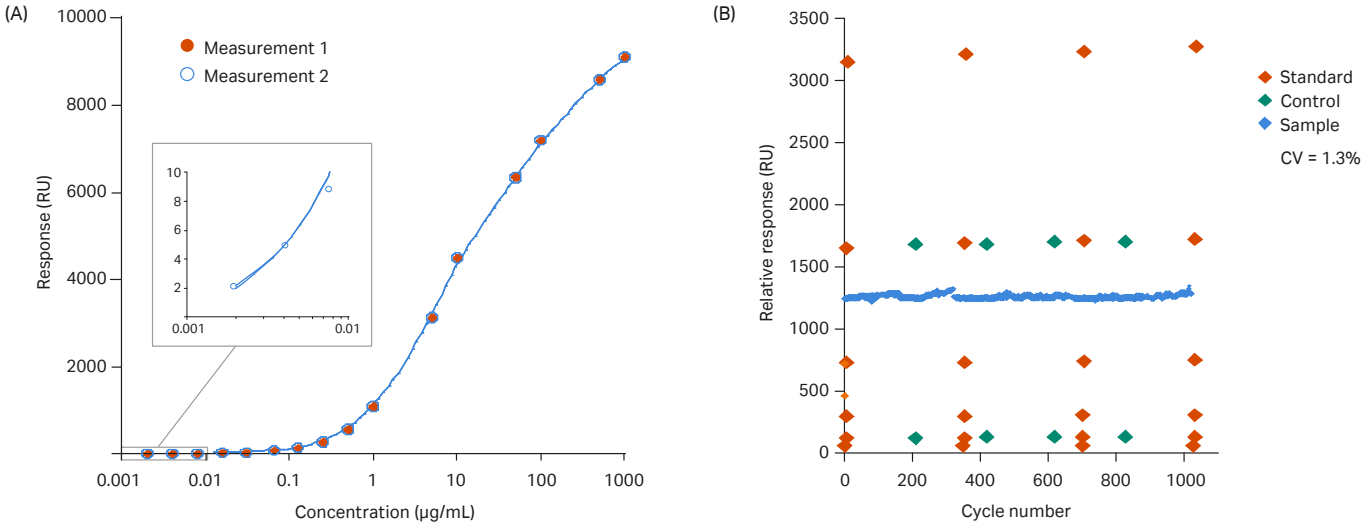


Fig 4. (A) Omalizumab at concentrations from 2 ng/mL to 1 mg/mL was injected for a 3 min period. The insert shows the response for the lowest (2 to 8 ng/mL) concentrations. (B) The figure shows data obtained with repeated injections of standards, controls, and one selected sample over 1000 analytical cycles using the same surface. Noticeably, standard and control samples were stable over time and the coefficient of variation for the repeat sample was 1.3%.

Biacore binding assays reflect the mechanism of action and can be developed into potency assays

Target binding

Antibodies, cytokines, and hormones typically interact with their receptors. Cytokines and hormones typically retain their natural sequence but can be engineered for improved stability or half-life. Antibody therapeutics on the other hand are engineered to interact with relevant target molecules including antigens, Fc receptors, and complement factors. The binding profiles presented in Figure 5 illustrate the binding of a cytokine to its receptor and two antibodies binding to their targets.

Clearly, binding properties vary considerably with estimated half-lives from 50 s (IFNα-2a) to 12 h (bevacizumab). Today there are several antibody formats in development with particular focus on bispecific antibodies where two distinct target functionalities are combined in one molecule. Examples of the use of Biacore SPR analysis for characterization include bispecific antibodies that bridge T cell and target-cell receptors (31); a bispecific antibody bridging factor IXa and factor X binding to mimic the natural function of factor VIII (32); and a bispecific antibody that combines vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang-2) functionalities to reduce the formation of blood vessels in cancer tissue (33). Target binding is clearly an essential CQA and must be controlled during development and later in QC for batch-to-batch consistency. While release assays are traditionally based on bioassays, ligand-binding assays (22, 34) can be considered when the mechanism(s) of action is/are defined provided that the assays reflect the mechanism of action (MOA). While kinetic data is useful for in-process characterization and batch-to-batch comparison, release assays require that the ligand-binding assays produce a product concentration that reflects the pharmacological activity (potency assay). Release assays are often based on relative comparisons and parallel line/parallel logistic analysis with defined conditions for equivalence (35).

The dual-specificity assay for VEGF-Ang-2 antibodies described in (30) was later developed into a potency assay (23) as illustrated in Figure 6. VEGF was immobilized to the sensor surface. The bispecific Ang-2/VEGF CrossMAb (Roche) was injected followed by a second injection of angiotensin (Ang-2, Fig 6A). Two response values, R1 and R2, corresponding to VEGF and Ang-2 binding, respectively were obtained (Fig 6B). R2 values were further plotted vs the logarithm of the concentration of the CrossMAb (Fig 6C) and similar plots were obtained from experiments using deviating concentrations of the CrossMAb and stressed samples. Parallel line analysis (PLA) of these curves showed that the assay was applicable for potency estimates in the range from 60% to 140% of the nominal concentration.

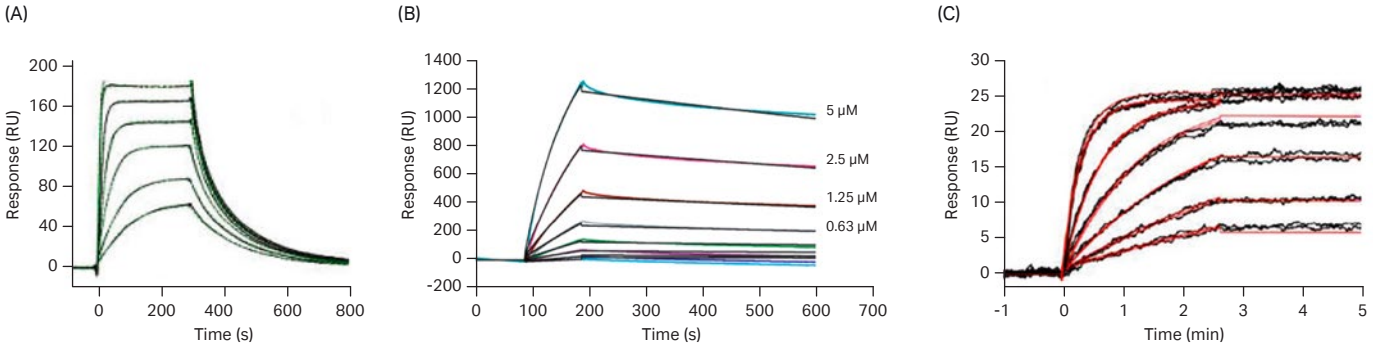


Fig 5. Binding of (A) interferon alpha 2a (IFNα-2a) to IFNAR2 (28), (B) CD20 to rituximab (29), and (C) vascular endothelial growth factor (VEGF) to bevacizumab (30). The plots are overlay plots of measured data and kinetic binding curves obtained with a 1:1 binding model. Reprinted (adapted) with permission from: (Fig 5A) Dhalluin C, Ross A, Huber W, *et al*. Structural, kinetic, and thermodynamic analysis of the binding of the 40 kDa PEG-interferon-alpha2a and its individual positional isomers to the extracellular domain of the receptor IFNAR2. *Bioconjug Chem.* 2005 May-Jun;16(3):518-27. doi: 10.1021/bc049780h, reference 28; (Fig 5B) Ernst JA, Li H, Kim HS, Nakamura GR, Yansura DG, Vandlen RL. Isolation and characterization of the B-cell marker CD20. *Biochemistry*. 2005 Nov 22;44(46):15150-8. doi: 10.1021/bi0511078., reference 29; (Fig 5C) Papadopoulos N, Martin J, Ruan Q, *et al*. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. *Angiogenesis*. 2012 Jun;15(2):171-85. doi: 10.1007/s10456-011-9249-6., supplementary material from electronic article, reference 30. © 2015 American Chemical Society.

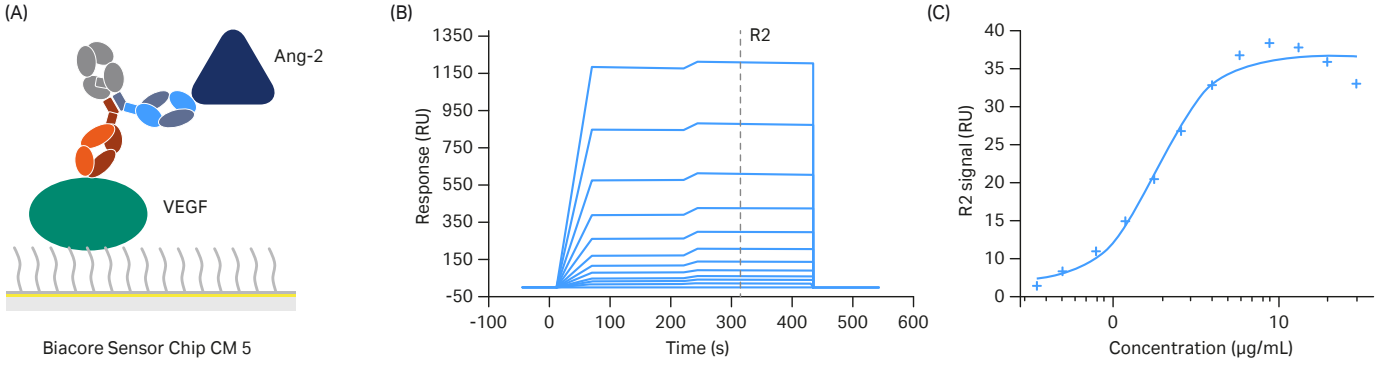


Fig 6. Potency assay setup for VEGF-Ang-2 CrossMAb. (A) Interaction of Ang-2 and VEGF with bispecific CrossMab analyzed by SPR using a Biacore system. (B) Overlay of sensorgrams recorded with CrossMab concentrations of 0.35 to 30 g/mL (1:1.5 dilution series) with 1 g/mL Ang-2 binding. (C) Dose-response curve (Ang-2 binding response R2 vs CrossMab concentration, log scale) of the CrossMab binding signal. Reprinted from Gassner C, Lipsmeier F, Metzger P, *et al*. Development and validation of a novel SPR-based assay principle for bispecific molecules. *J Pharm Biomed Anal.* 2015 Jan;102:144-9. doi: 10.1016/j.jpba.2014.09.007., reference 25. With permission from Elsevier.

Fc receptor binding

Fcγ receptors (FcγR) are expressed on different cell types and can be activating (FcγRI, FcγRIIa, and FcγRIIIa), inhibitory (FcγRIIb), or without effect (FcγRIIIb) in antibody-dependent cellular cytotoxicity, ADCC. The mechanism of action for several anticancer antibodies involves ADCC and here the interaction with FcγRIIIa present on natural killer cells might be of particular importance. Biacore binding analysis is widely used in research where antibodies are designed either for improved interaction with FcγR (36) or for elimination of immune effector functions wIhen non-immunostimulatory monoclonal antibodies (MAb) are developed (37).

The neonatal Fc receptor (Fc receptors) interacts with antibodies at a lower pH and is believed to be important for rescuing antibodies from lysosomal degradation and for prolonging the half-life of antibodies (38, 39). In late-stage development, the objective is to maintain the established FcγR and Fc receptors functions and in this context, Biacore SPR assays are widely used in comparability studies (12). Several assay formats have been used for studying the Fc receptors-antibody interactions including: covalent coupling of Fc receptors (40, 41, 42); capture of histidine-tagged Fc receptors on immobilized anti-histidine antibody (37, 43, 44); and capture of antibodies on protein L (45) and protein A (46).

Capture formats combine the advantages of the orientation of the captured molecule and common regeneration conditions across a range of interactions. Two capture formats used for FcγR analysis are illustrated in Figure 7A. Both these formats give stable capture and highly reproducible data and can be used across the entire range of FcγR with no change to the assay setup, thereby facilitating fingerprint analysis as illustrated in Figure 7B. This fingerprint of rituximab-FcγR interactions is characteristic for rituximab.

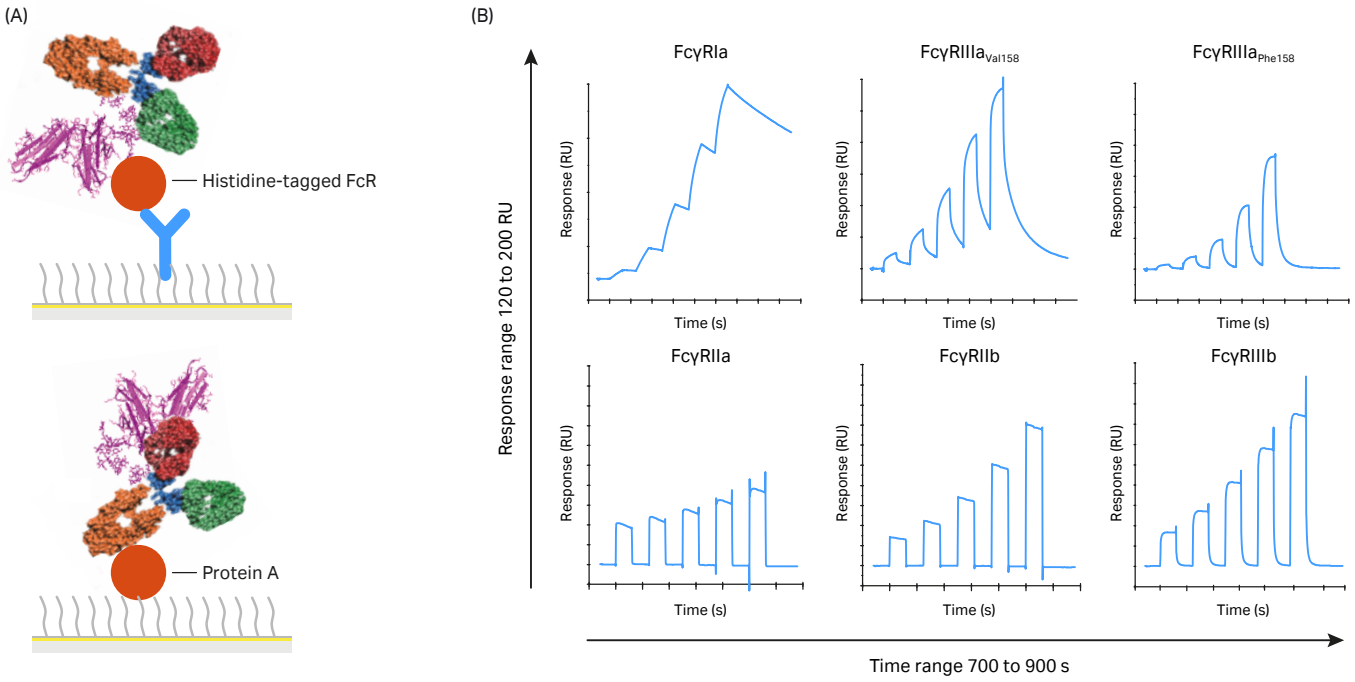


Fig 7. (A) Two FcγR assay procedures based on capture are illustrated. Top panel: immobilized antihistidine antibody capture of histidine-tagged FcR and binding of antibody. Lower panel: Coupled protein A, capture of antibody and binding of FcR. (B) Binding profiles for rituximab binding to a range of FcγR. Reprinted (adapted) with Hayes JM, Frostell A, Cosgrave EF, *et al*. Fc gamma receptor glycosylation modulates the binding of IgG glycoforms: a requirement for stable antibody interactions. *J Proteome Res.* 2014 Dec 5;13(12):5471-85. doi: 10.1021/pr500414q., reference 43. © 2015 American Chemical Society.

Fc receptors interactions are typically studied at low (~ 6.0) and high (~ 7.4) pH to mimic conditions where Fc receptors protects the antibody from lysosomal degradation (low pH) and where the antibody can be released on a cell surface (high pH). The importance of Fc and Fab structures for Fc receptor binding has been intensively studied. MAb with identical Fc sequences can bind to Fc receptors differentially (44) and antibodies pre-incubated with antigen bind tighter to Fc receptors (36). Fc receptors assays are usually set up with immobilized receptors, and for reproducible results, maintaining strict assay conditions is recommended as results can be influenced by the level of the immobilized receptor (40). Typical binding curves at low pH and release curves at high pH are illustrated in Figure 8.

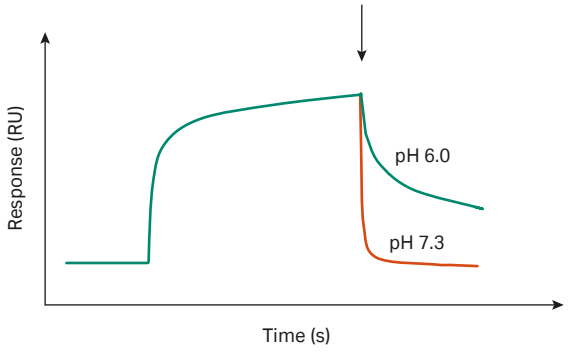


Fig 8. Binding and dissociation of antibody to FcRn at pH 6.0 (green) and dissociation of the antibody as pH is shifted to 7.4 (orange).

While Fc receptors assays with Biacore systems are essential for antibody characterization, this approach has also been proposed as a potency assay for antibodies where the interaction with FcγRIIIa contributes to the mechanism of action. Harrison and co-workers (44) used histidine-tagged receptor and alemtuzumab to develop a potency assay based on report points from several concentrations of the antibody. The potency assay used the 5PL algorithm and demonstrated good linearity and measured potencies were within 9% of the nominal values. The authors noted that the SPR assay showed greater internal precision and long-term reproducibility than a traditional cell-based ADCC assay.

Binding of complement cascade C1q to antibody

The first step in the complement cascade is the binding of C1q to the antibody (48). The C1q molecule has six heads connected by collagen-like stems to a central stalk. The isolated heads bind to the CH2 domain of the antibody. Blanquet-Grossard and co-workers (49) have demonstrated IgG binding to immobilized C1q. In Figure 9, the sensorgram is indicative of a slow on-rate and a slow off-rate. Note that the concentration of antibody is 1.5 µM and that the binding rate is low. Although these results indicate that C1q assays on Biacore systems are feasible, more public data on this assay would be helpful.

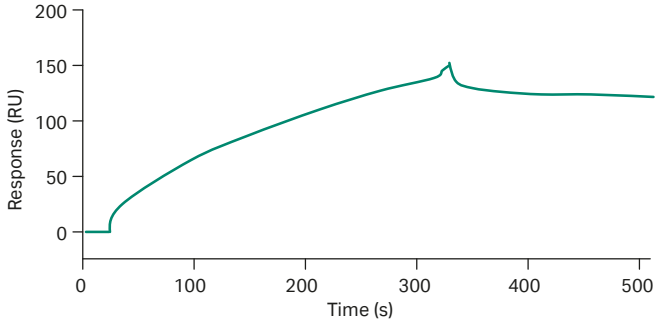


Fig 9. Binding of 1.5 µM rabbit IgG to attached C1q. Reprinted (adapted) with permission from Blanquet-Grossard F, Thielens NM, Vendrely C, Jamin M, Arlaud GJ. Complement protein C1q recognizes a conformationally modified form of the prion protein. *Biochemistry*. 2005 Mar 22;44(11):4349-56. doi: 10.1021/bi047370a, reference 49. © 2015 American Chemical Society.

Biacore systems in stability and forced degradation studies

Changes in higher order structure can potentially alter the immunogenic profile of a biotherapeutic drug and thereby impact the safety of the drug. Amino acid modifications and changes in glycosylation can be identified by a combination of analytical techniques such as MS, electrophoresis, and chromatography and product variants can be isolated and characterized.

Methionine oxidation, glycation, and deamidation can be induced by protein stress. Whether such changes are actually accompanied by a change in conformation is uncertain. Interestingly, not only amino acid modifications but also 25% to 50% reduction in target binding, as determined by a Biacore assay, could be observed for stress conditions related to elevated temperature, elevated pH, and presence of hydrogen peroxide (50). Similarly, methionine oxidation of an IgG2 resulted in impaired binding to Fc receptors (51). Changes in target binding and Fc receptors binding for stressed samples thus indicate that interaction analysis may be used to detect small changes in antibody reactivity. Target and Fc receptors interactions can therefore play an important role in stability and forced degradation studies.

To obtain more comprehensive fingerprints of antibody reactivity, we identified several domain-specific antibodies and bacterial proteins that are capable of binding IgG as well as differentiating between wild-type (WT) and stressed variants. In Figure 10, the stressed variant was subjected to treatment with 0.2% hydrogen peroxide for 18 h. MS analysis demonstrated a 20-fold increase in methionine oxidation increasing from 1% to 2.5% to between 20% and 50% depending on methionine position. Four common IgG binders directed towards Fc and hinge regions were able to differentiate between wild type (WT, green curves in Fig 10) and forced oxidized samples (orange curves). Similarly, three reagents identified changes from pH stress leading to deamidation and two reagents differentiated between WT and light-stressed antibodies. The latter were more prone to aggregation and compared to WT demonstrated differences in hydrophobic and charge profiles.

This data suggests that Biacore systems can be used for rapid analysis of changes in binding reactivity related to target-, Fc receptors-, and IgG domain-specific functionalities and strengthens the use of Biacore SPR analysis for screening of changes in reactivity in forced degradation studies and in regular stability testing.

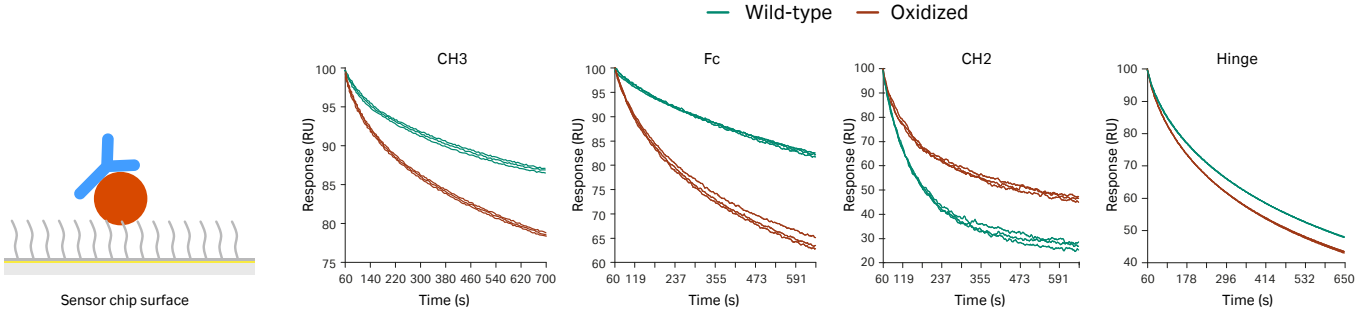


Fig 10. Antibody reagents reacting with Fc and hinge regions bind differently to oxidized samples than to WT variants. Dissociation of the stressed antibody differed from that of the WT. For one reagent reacting with the CH2 domain, the dissociation rate was reduced and with other reagents, more rapid dissociation was observed with the stressed antibody.

Surrogate Biacore potency assay (EC50) for comparison of binding profiles to complement dose response curves

Potency estimation is critical for the release of each lot of a therapeutic antibody (54). Most of the release assays are based on bioassays, but alternatives such as ligand binding assays are being used in recent years (34). The potency assay is not only used as a release assay but can also be used throughout the development process in comparability studies and formulation studies to ensure consistency between drug substance and drug product.

Traditionally, PLA and EC50 analysis on SPR platforms have been focused on comparison of dose response curves. One of the published articles from our Biacore research and development team (55), illustrates new possibilities with Biacore SPR assays for binding activity measurements. It describes the use of reversible biotin capture to establish dose-response curves for the measurement of relative potency via PLA and EC50 analysis and illustrates this using antitumor necrosis factor (TNF)-α antibodies (Fig 11). It demonstrated the estimation of dose response using Biacore SPR-based EC50 analysis and therefore relative potency determinations. Here, the Biacore SPR assay is used to understand the sensitive changes in active concentration but also to changes in binding properties. Such changes may not always be compensated by adjustment of the dose. For instance, large differences between the reference and sample in dissociation properties, which may go undetected in dose-response curve analysis, can impact drug residence time, and therefore potentially affect the drug efficacy. By complementing the dose-response curves with sensorgram comparisons, such deviations, can be detected as sensorgram comparison checks for compliance with kinetic properties. The sensorgram comparison analysis compares reference and sample curves directly and can be applied to both simple and complex binding data and can be used even with slow off-rates (56).

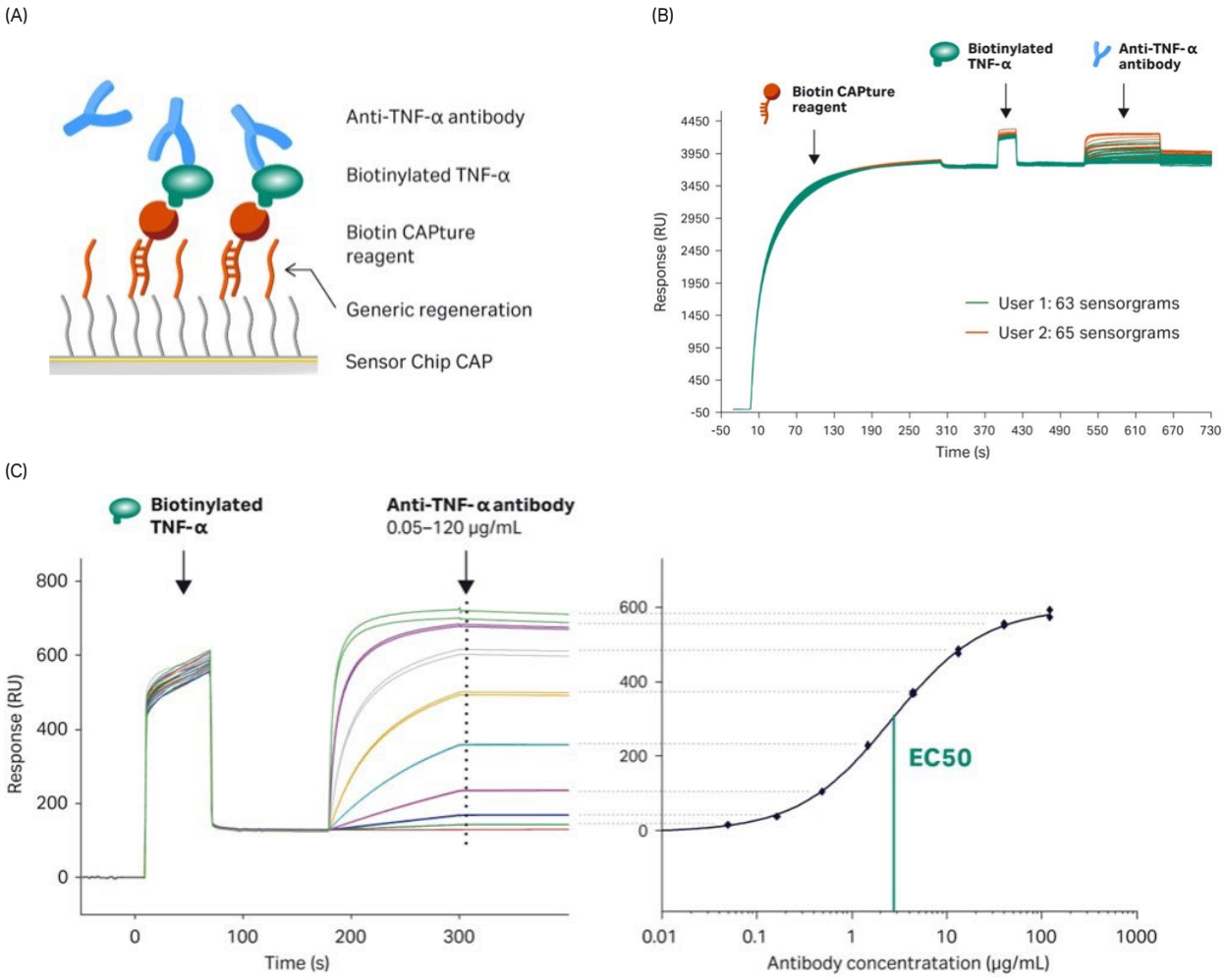


Fig 11. (A) Biotin capture assay setup on Biacore sensor chip to study the potency assay. (B) In the biotin capture assay, the biotin capture reagent (streptavidin modified with an oligonucleotide) is first injected and hybridizes to the Sensor Chip CAP containing a complementary oligonucleotide. Biotinylated TNF-α is then injected followed by the injection of an anti-TNF-α antibody at varying concentrations. The figure is an overlay plot of 128 analytical cycles and shows binding events in the active flow cell. The color coding refers to two users. (C) Left image shows the anti-TNF-α antibody injected at varying concentrations (0.05–120 µg/mL), binding to biotinylated TNF-α captured on the sensor chip, and right image shows its potency (EC50 analysis) determination.

This article also described the use of calibration free concentration analysis (CFCA) (57, 58) as an alternative to potency assay (EC50) for the analysis of stressed anti-TNF-α antibody samples. These studies can help in establishing the links between molecular properties and clinical effects, and ligand binding assays may be more frequently used not only for comparability and biosimilarity studies, but also for batch release.

Biacore systems in comparability and biosimilar studies

Sensorgram comparison for objective evaluation of Biacore binding curves

Kinetic and affinity analysis is very useful for comparison of interaction data. Nonconformance in kinetic and affinity parameters from one sample to another clearly signals that a change has occurred. However, kinetic and affinity analysis assumes that the interaction can be described by a binding mechanism and that a model can be fitted to observed data to obtain kinetic and affinity parameters. As illustrated by the excellent fits in Figure 5, this is often the case. However, the binding mechanism is not always known, interaction partners can be heterogeneous, or kinetic data can be uncertain. The interaction between rituximab and FcγRIIIa$_{Val158}$ illustrated in Figure 12 is a good example.

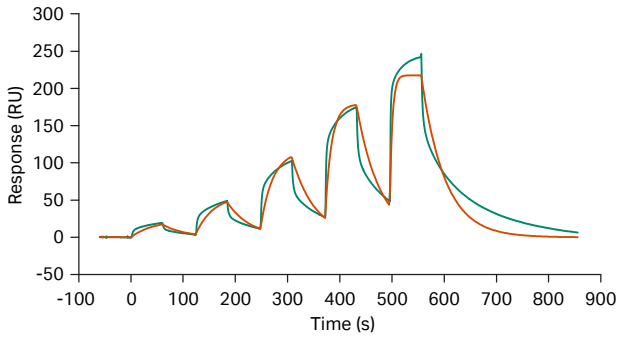


Fig 12. Binding of rituximab to FcγRIIIa$_{Val158}$ (green curve) and data fitted to a 1:1 binding model (orange curve).

This interaction cannot be fitted to a simple interaction model and a detailed analysis reveals that the complexity is related to both receptor and antibody heterogeneity (43). However, the assay is reproducible and when interaction data obtained using varying receptor levels (Fig 13A) is normalized with respect to the amplitude of the response, binding shapes become similar (Fig 13B). In Figure 13C, a response window defined by the average sensorgram ± three standard deviations has been created. A new sample of rituximab can now be assumed to fall largely within this window. If all data points are within the standard deviation window, the new sample obtains a similarity score of 100%. If data points fall outside the assay window the similarity score is reduced and the farther away the data is from the window, the higher the penalty will be. This statistical approach to sensorgram analysis makes it possible for you to compare sensorgrams even when the binding mechanism is unknown, when the interaction is too complex to be modeled, or when there is a large uncertainty in reported rate constants.

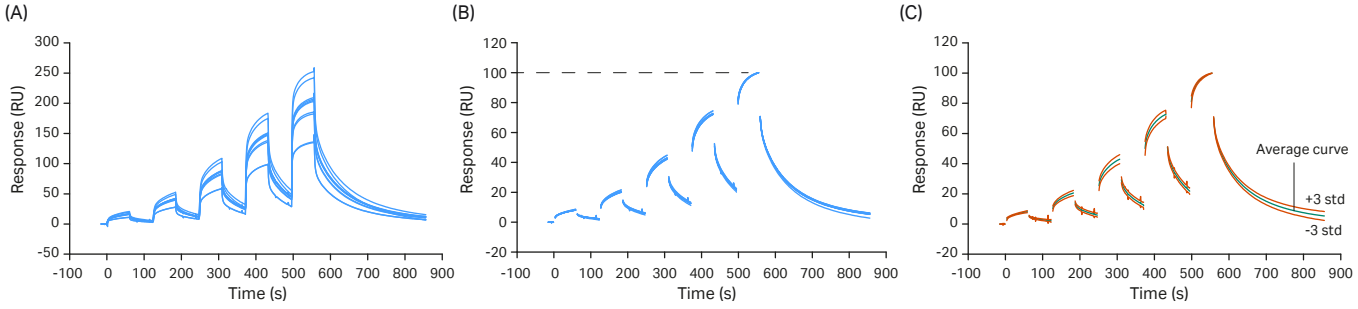


Fig 13. Binding of rituximab 25 to 2000 nM to FcγRIIIaVal158. (A) Binding data obtained with different capture levels, (B) normalized binding data, (C) a response vs time area defined by average and standard deviation sensorgrams.

When infliximab, omalizumab, and trastuzumab binding to FcγRIIIa$_{Val158}$ was compared to that of rituximab, infliximab obtained a similarity score of 75% while the humanized antibodies (omalizumab and Herceptin trastuzumab) obtained similarity scores close to 50% (Fig 14). With a robust assay, the sensorgram comparison approach provides a convenient alternative for the comparison of binding data and can prove useful in batch-to-batch comparisons in comparability and biosimilar studies. An advantage of the sensorgram comparison approach is that calculations are rapid and that no fitting procedures must be used. It is however important to carefully define the standard data that are used for the calculation of the variation window as scoring is always with respect to the selected standard.

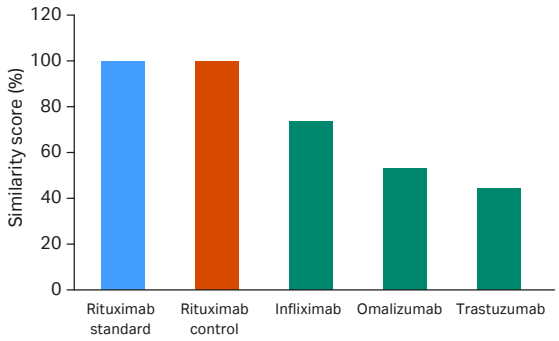


Fig 14. Similarity score for rituximab standard (blue bar), rituximab control (orange bar), infliximab, omalizumab, and trastuzumab binding to FcγRIIIa$_{Val158}$.

Calibration-free concentration analysis for rapid absolute and relative concentration measurements

Concentration analysis based on ligand binding typically requires a standard preparation with known active concentration. However, a standard preparation might not always be available, for example, when a protein is expressed for the first time or when concentration data for the standard reflects the total protein concentration and not the active concentration. In such circumstances, Biacore analysis can be used for direct assessment of the active concentration (58). This possibility was described already in 1993 (51) and has been refined with modern numerical integration tools for data analysis (53). The calibration-free concentration analysis (CFCA) method illustrated in Figure 15 provides a good estimate of the absolute concentration assuming that the diffusion coefficient of the analyte is known and that the observed binding rate is flow-rate dependent (57).

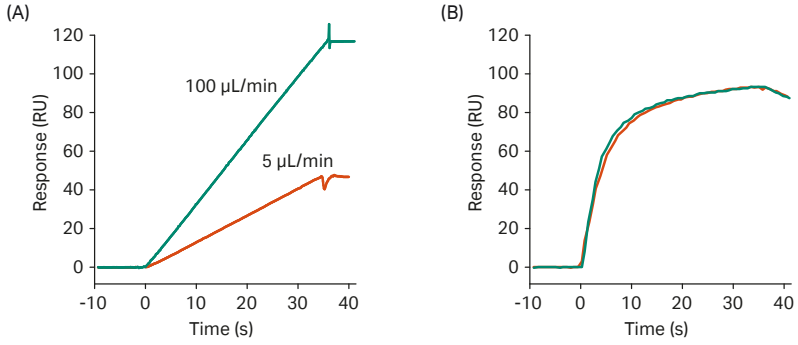


Fig 15. CFCA is based on initial binding rates. If binding rates are flow-rate dependent (as in A), CFCA is possible but when binding rates are flow-rate independent (as in B), CFCA is not possible.

CFCA is an excellent tool for comparison of your concentration data and is particularly useful for reagent characterization. CFCA is also a complement to kinetic analysis and sensorgram comparison methods when these indicate that changes in the interaction can be related to changes in active concentration. The use of CFCA for analysis of chromatography fractions and to guide purification efforts is described for you in reference 59. Considering that relative concentration data is so easy to obtain with CFCA, it may also be used to complement and support potency assays.

Biacore systems are tailored for work in a regulated environment

Regulatory authorities impose increasing demands on pharmaceutical development and manufacturing companies to use quality-assured equipment and to follow detailed and carefully documented analytical and manufacturing procedures. The purpose of this control effort is to ensure consistent and reliable quality in pharmaceutical products that reach the market.

Biacore GxP package offers you qualification services in connection with installation and service maintenance of instrument, to provide the user with the necessary documentation for equipment qualification. Furthermore, Biacore Insight GxP Extension offers you the possibility to add user authorization levels where administrator, developer, and users set access rights to software functions.

Within Biacore Insight GxP extension software, the groups are permitted access as follows:

Administrator: Full access to system operation and evaluation. Certain settings related to folder settings in the software are only accessible to administrators, who therefore have the responsibility of determining these settings for all other users.

Developer: Access to all functions in system operation and evaluation except for software preferences. developers are responsible for developing and publishing procedures for routine use. Published procedures consist of a run method and an evaluation method. This ensures that data is generated and evaluated according to defined protocols.

User: Access only to published procedures for system operation and data evaluation and to control software functions required for the operation and maintenance of the instrument. Users may only perform runs based on published procedures. In evaluation software, users may only open results and evaluation files derived from published procedures. Some flexibility exists for the user, but changes made to the contents of result and evaluation files derived from published procedures are logged in in an operator-independent audit trail.

Summary

The life cycle of a biotherapeutic agent can extend over 20 to 30 yr. During this time, you must develop and deliver a product with consistent quality. In support, regulatory authorities such as the FDA and EMA issue guidelines aimed at ensuring the efficacy of biotherapeutic medicines. Antibodies, cytokines, and hormones assert their actions through interactions with their target molecules and ligand-binding assays are highly relevant for the characterization of the drug.

Biacore SPR systems are used specifically in drug development to define CQA and for concentration analysis in cell culture, purification, and formulation workflows. In addition, the systems perform kinetic analysis of drug-target interactions, Fc receptor analysis, and assessment of changes in reactivity that can occur in stressed samples. You can use combined interaction data for fingerprint analysis to provide a broader perspective of how a drug interacts with its partners and how stable it is under conditions of stress.

SPR is a direct-binding technique that can measure sequential binding events. This lets you perform analysis of dual-target specificities in a bispecific antibody in a single assay setup. Potency assays for drug-target interactions and Fc receptors interactions in QC are described in this white paper.

Sensorgram comparison and CFCA methods provide you with novel opportunities in comparability and biosimilar studies and have implications for potency assays.

Biacore systems have been developed to function in a regulated environment and come with installation and maintenance support and with software that combines flexibility in development with rigor in a QC environment. The product offering from Cytiva includes Biacore systems and consumables in the form of sensor chips and capture kits to simplify development and make your binding assays easy, and reliable, with a streamlined transfer of methods from one site to another.

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