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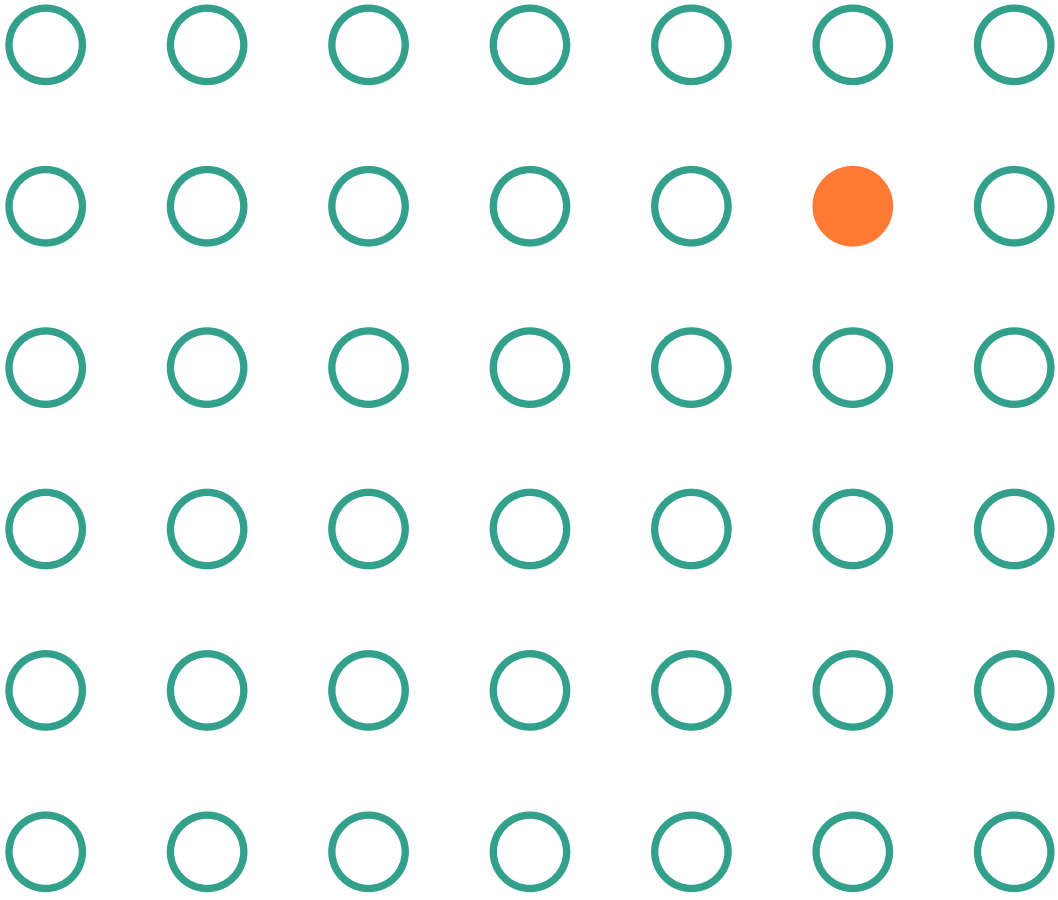
A translational perspective: Biacore™ systems in discovery and early-stage development of biotherapeutic antibodies



Abstract

Translational science has launched a new era of antibody therapeutics, driving demand for novel antibody formats designed to improve efficacy of therapies and reach new targets. The trend towards increasingly complex drug targets drives the need for increased sensitivity during development, screening, and lead optimization. Biacore™ surface plasmon resonance (SPR) systems are extensively used in biotherapeutic antibody discovery and development. Here, we discuss the utility of Biacore systems from selection of first candidates to clinical lead. We show that a combination of Biacore SPR systems, software, sensor chips, and kits support the setup of screening and characterization assays and reduce the difficulties that come with assay development. During screening, antibody capture followed by an antigen injection permits the selection of monophasic and stable binders with preferred kinetics and stoichiometry. Standardized Biacore epitope binning procedures ensure reliable determination of epitope specificity, while the effect of antibody engineering efforts can be investigated by analysis of antibody binding to antigen and Fcγ-receptors. Biacore SPR systems also address key developability aspects, including ensuring critical binding properties remain unchanged in forced degradation studies. While using Biacore SPR systems, antibody concentration and kinetics can be monitored in the presence of nonbinding unfolded fractions, host cell proteins, and other impurities. **For translational scientists seeking to progress their drug discovery from benchtop to clinical testing and beyond, Biacore SPR systems can provide the reliability, sensitivity, and automation to reduce risk of failure and assist with a smooth journey to clinic.**

Introduction

Over the last 30 years, recombinant proteins including hormones, cytokines, and therapeutic antibodies were developed by academics and advanced by translational scientists for the treatment of diseases including diabetes, cancer, and rheumatic disorders. Recombinant insulin in various forms is a commonly prescribed biotherapeutic medicine. While hormones and cytokines represent an important class of biotherapeutics, antibodies are now the focus and will eventually have a wider applicability. The number of FDA approved antibodies is steadily increasing (1), demonstrating that the translational science space is accelerating drug discovery, with approved antibodies now directed at over thirty different target molecules. In 2021, the FDA approved the 100th monoclonal antibody product. (Fig 1). Other emerging areas of translational medicine include; antisense oligonucleotides, mRNA- based drugs, and targeted protein degraders. In this white paper, we outline the value of Biacore SPR system technology to the translational scientist concerned with antibody discovery and development.

From antibody generation to clinical lead: the value of developability assessments

Modern techniques for antibody generation often yield numerous candidates that achieve desired functional properties. This can make it challenging for translational scientists to select a lead candidate for downstream resources and labor-intensive steps such as cell line development, process development, and formulation. Assessing a candidate's developability — the likelihood of successfully developing a lead candidate into a stable, manufacturable, safe, and efficacious drug — is highly advantageous. **By interrogating lead candidate developability aspects early, both the success rate and speed of preclinical and clinical development can be enhanced.** Liabilities like product heterogeneity, stability, and unwanted *in vivo* effects are avoided.

The antibody development workflow (Fig 1) has evolved, with early development no longer focused solely on potency and functional aspects such as specificity, affinity, and kinetics for molecular targets. The developability aspects play an increasingly important role for reducing risk of failure later in the development program (2).

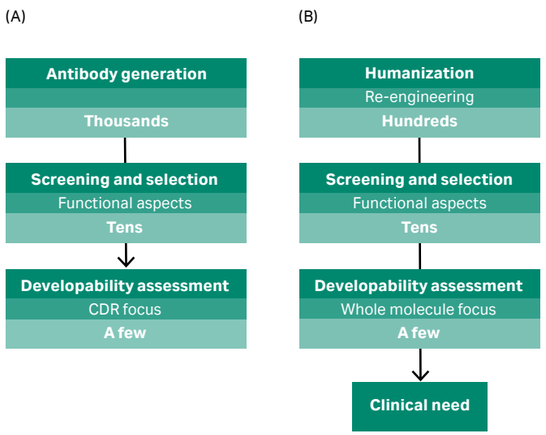


Fig 1. The antibody development workflow. (A) First selection round with focus on antigen binding, and (B) Re-engineering and selection of clinical lead.

During antibody development (Fig 1), there are two stages where developability assessments can be performed. At each assessment there will likely be multiple promising candidates selected for further development. A thorough characterization of biophysical antibody properties is not always necessary and, to save time and costs, it can be beneficial to focus on the most prevalent liabilities to eliminate candidates.

These include:

- Examining complementary determining regions (CDRs) for potential degradation sites (2).
- The impact of post-translational modifications (PTMs), for example, methylation, acetylation, phosphorylation, glycosylation on stability and conformation (3,4).
- Aggregation and fragmentation tendencies (5).
- Solubility and solution stability (6).
- Biological factors such as immunogenicity (7) and pharmacokinetic properties (8).

The core questions that translational scientists seek to answer during developability assessment are:

Can the antibody be manufactured?

While a candidate lead antibody may show high affinity, potency, and specificity — if the manufacturing process or physical properties are suboptimal, it may be unsuitable for production at therapeutic scale. Suboptimal characteristics can provide difficulty in streamlining and accelerating the manufacturing process because monetary investments may be required to implement additional downstream processes to move the molecule forward into development. Preferred routes of administration may not be achievable if the molecules cannot sustain therapeutic concentrations because of aggregation or viscosity (9). Key manufacturability assessment attributes include purity/heterogeneity, stability, solubility, upstream/downstream processing, and formulation. Assessing these attributes enables translational scientists to achieve their key manufacturability endpoints, including long-term stability in formulation buffer and viscosity at high protein concentrations.

Is it safe?

Since antibodies directly interact with the immune system, many carry an inherent risk of adverse immune-related reactions. Some promising lead candidates fail because of toxicity to humans. Detecting these issues early in the selection process is highly favorable. This includes assessing immunogenicity/immunotoxicity, affinity for known toxicological targets, off-target binding, and half-life.

Will it have acceptable bioavailability and efficacy?

A lack of efficacy is one of the primary reasons why drugs fail at clinical trials. Ensuring that an antibody lead candidate displays a satisfactory level of bioavailability and efficacy is vital to success further down the line. Selecting lead candidates with optimal affinity, potency, specificity, and pharmacokinetics leads to better success in selecting a high-efficacy candidate.

New antibody formats

A large majority of FDA approved antibodies are full-length and of IgG1, IgG2, or IgG4 subclass. However, non-traditional antibody formats are slowly emerging. For example, blinatumomab (Blincyto, approved 2014) is a bispecific antibody while brentuximab vedotin (Adcetris, approved 2011) and ado-trastuzomab emtansine (Kadcyla, approved 2013) are based on conventional antibodies but conjugated with cytotoxic agents, and are so-called antibody-drug conjugates (ADCs). New formats are introduced to improve the efficacy (10) of therapies to reach new targets, for instance by designing antibodies that have the capability to cross the blood-brain barrier (11). Antibody formats that retain the basic structure of IgGs may inherit their pharmacokinetic properties (12) but novel constructs that lack the Fc-part of the antibody may have reduced half-life (13). This can be an advantage if the antibody is used for imaging purposes (14). For therapeutic purposes, there needs to be a balance between efficacy and half-life for smaller antibody formats such as single chain Fvs and nanobodies, or other scaffolds that have the potential to reach more hidden targets and even act as intrabodies to target intracellular antigens (15). Several pharmaceutical companies now have bispecific antibodies (16) and antibody-drug conjugates in their clinical pipeline (17) while intracellular antibodies may still be in research phase (Fig 2).

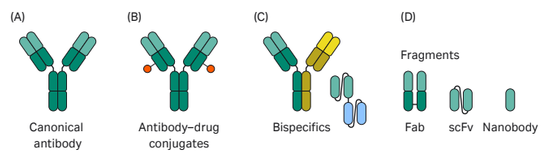


Fig 2. Antibody formats. Antibody formats include canonical (A), antibody–drug conjugates (B), bispecifics (C) and fragments (D). Fragments include antigen- binding fragments (Fabs), single- chain variable region (scFv) constructs, and domain antibodies. Radiolabelled antibodies and antibody–immunotoxins are not shown. These formats can be further subcategorized, and antibodies can span classifications. There are at least 30 different bispecific formats, for example, some of which include fragments. Modified from *Nature Reviews Drug Discovery*.

Efficiently select and optimize antibody candidates

Biacore system technology was incorporated in the antibody development workflow almost immediately after launch in 1990, when kinetic analysis of antibody-antigen interactions (18) and epitope binning procedures (19) were described. Antibody D2E7, which later became Humira, the bestselling therapeutic antibody in 2021 (20), was selected from a Biacore screen in the mid-1990s (21). From screening of candidates to antibody engineering and final development, Biacore systems have been consistently used to determine specificity of binding to characterize antibody-antigen and antibody-Fc receptor interactions and to guide development towards a clinical lead. In developability studies, Biacore systems are typically used to monitor effects of forced degradation on antigen and Fc gamma receptor binding and for assessment of pharmacokinetic properties where binding to FcRn is related to antibody half-life. More recently, the use of binding mode-specific reagents (22) has been described for detection of changes in antibody topography as a consequence of forced degradation.

Biacore systems in antibody development

Depending on the design of the flow system, direct interaction analysis with several target molecules can be performed with a single injection of sample. Typically, one measuring spot is used for active analysis and one for referencing (Fig 3). Depending on the Biacore system, one to eight active/reference pairs can be used. Independently of the number of active reference pairs, double referencing is typically performed to arrive at high quality data by subtracting blank cycles.

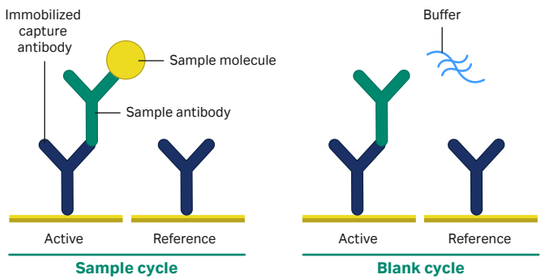


Fig 3. Active and reference measuring spots and their use in sample and blank cycles.

Sample throughput is linked to the number of microwell plates that can be handled in an automated run. Higher throughput systems are suited for antibody screening and large-scale epitope binning experiments while other systems have functions that make them ideal for detailed characterization studies. There is a considerable overlap in the systems and those intended for screening are also used for characterization and vice versa.

Minimize time spent on assay development with dedicated sensor chips and reagents

Antibody applications are supported by several sensor chips and reagents provided with ready-to-use protocols, enabling rapid assay development using well known and reversible capture formats.

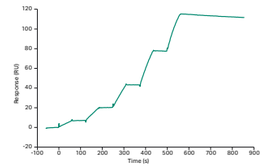
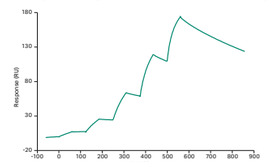
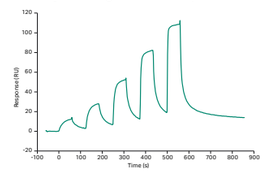
Products	Application example
Sensor Chip Protein A Sensor Chip Protein G Sensor Chip Protein L Sensor Chip PrismA	Antigen binding to captured antibody 
Capture reagents Histidine capture Anti mouse Fc Anti human Fc Anti human Fab Direct immobilization	Antibody binding to histidine captured FcgRI 
Reversible streptavidin Biotin CAPture kit	Antibody binding to biotin captured FcRn 

Fig 4. Biacore™ sensor chips and kits for reversible capture of biomolecules.

Biacore Sensor Chip Protein A, Sensor Chip Protein G, or Sensor Chip Protein L (Fig. 4A) can be used directly for antibody concentration measurements or for capture of antibodies and subsequent kinetic analysis of antibody–antigen interactions. Sensor Chip PrismA (Fig 4A) is suitable for antibody concentration measurements but not recommended for kinetic analysis of antibody–antigen interactions. Biacore Sensor Chip CM5 (Fig. 4B) can be used for direct immobilization and be combined with several antibody capture kits. With the Mouse Antibody Capture Kit, all IgG subclasses, IgM and IgA can be captured to immobilized polyclonal rabbit anti-mouse immunoglobulin. The Human Antibody Capture Kit includes a monoclonal mouse anti-human IgG (Fc) antibody capable of capturing all IgG subclasses. The Human Fab Capture Kit features a mix of monoclonal antibodies and capture Fab through kappa and lambda chains. ScFv antibodies may be captured using protein L or its variants (23). In cases where a small antibody fragment, antigen or an Fc-receptor is captured on the sensor surface the His Capture Kit can be used to capture histidine-tagged molecules using a monoclonal anti-histidine antibody. Biotinylated reagents can be captured onto Biacore Sensor Chip SA, Sensor Chip NA, or for a reversible biotin-streptavidin interaction, Biotin CAPture Kit (Fig. 4C) can be used.

Deeper insight into biotherapeutic characteristics with improved efficiency

Translational scientists are looking to conduct earlier assessment of expression levels, target specificity, and binding stability for clone selection. The number of samples from hybridoma cells or recombinant expression systems varies greatly but may range up to thousands. A multi-step approach is often applied as shown in Figure 5.

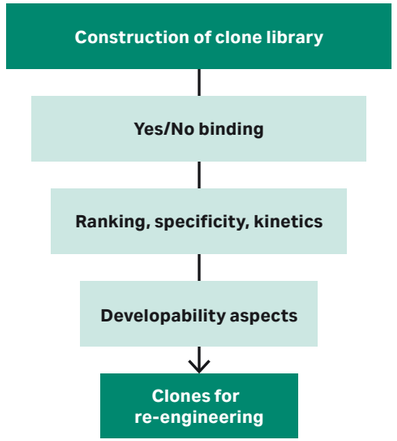


Fig 5. A stepwise approach to antibody selection. The clone library is analysed to allow selection of candidates for re-engineering.

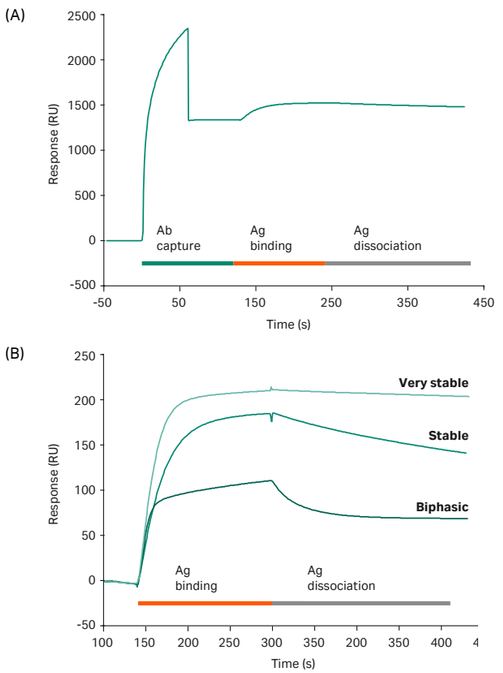


Fig 6. Screening data. (A) Assay setup with capture of antibody from media and injection of antigen, and (B) Comparison of different antigen binding profiles for identification of stable, very stable, and biphasic binding properties.

During these stages, samples are often crude and of limited volume with ELISA-based methods commonly used in screening to provide end-point results. Biacore systems can monitor and quantitate the entire interaction, and samples from cell culture supernatants can be directly injected (24). With the assay setup described in Figure 6, injections for capture of antibody and antigen binding are made in sequence (Fig. 6A). The antibody capture step provides information related to the expression level/concentration of the antibody and the second injection provides information on the rate and stability of the interaction with antigen. The antigen binding level may provide stoichiometric information and a closer inspection of the buffer flow phase may reveal whether the interaction is monophasic or biphasic (Fig 6B). With hundreds to thousands of sensorgrams, it may be convenient to condense the result into plots of report points (Fig 7). In Figure 7A, the position of two regions — stability early and stability late — are highlighted in the sensorgrams. In Figure 7B, a plot of the response levels associated with these regions is used to rapidly identify binders with slow off-rate. In this way, Biacore systems are capable of yielding high-content information with single injections of antibody and antigen. Data analysis is straightforward and can be focused directly to the desired binding properties.

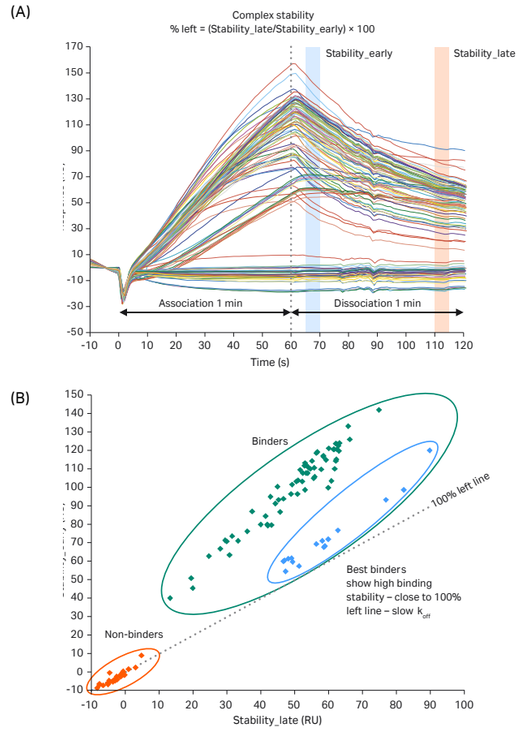


Fig 7. Sensorgram and report point analysis. (A) Antigen binding to antibody is presented in overlay plots. Two regions: stability early and stability late are indicated. (B) Response levels associated with the stability early region is plotted versus the response level from the stability late region. If the values are identical no dissociation has occurred, and the most stable binders are found close to the 100% left line.

Epitope binning for selection of appropriate specificities

Antigen binding is the first selection criterion, and must be accompanied with knowledge about epitope specificity (25) and whether the antibodies can assert the desired biological effect. Epitope binning experiments serve to identify and group antibodies with similar epitope specificity. Epitope binning experiments (26) are easily performed on Biacore systems by testing the selected antibodies against each other in a combinatorial setup. The experimental design includes capture of the first antibody, blocking of free antibody binding sites, injection of antigen, and finally injection of the second antibody. The assay setup and a corresponding sensorgram are schematically shown in Figures 8A and 8B and the result matrix from an eight-by-eight mapping experiment is shown in Figure 8C (27).

The result matrix is based on user-defined thresholds for antibody and antigen binding. When both antibodies can bind and no blocking occurs between them, the cells are white. Red cells indicate that antibodies share the same or have overlapping epitopes. In cases where the binding responses are too low or antigen dissociates too rapidly from the first antibody, mapping information may be uncertain, shown as yellow cells. These pairs can be revisited in a second experiment using longer injection times, or if antigen dissociates rapidly, the dual injection command can be used to inject the second antibody directly after the antigen injection with zero dissociation time. When binding of an antibody is blocked only in one direction, that is, when antibody A blocks binding of antibody B but not the other way around, their interaction is said to be uni-directional.

Uni-directional blocking is illustrated with arrows in the affected cells in the heat map. In this way it is possible to obtain high-resolution maps. Antibodies with shared or overlapping epitopes may be candidates for therapeutic use provided they exhibit the expected biological effect while antibodies directed towards different epitopes may be candidates for a sandwich assay. To identify epitopes more precisely, inhibition mapping (20) can be performed by pre-incubating the antibody with antigen domains or antigen-derived peptides and observing whether these constructs inhibit antibody binding to the antigen.

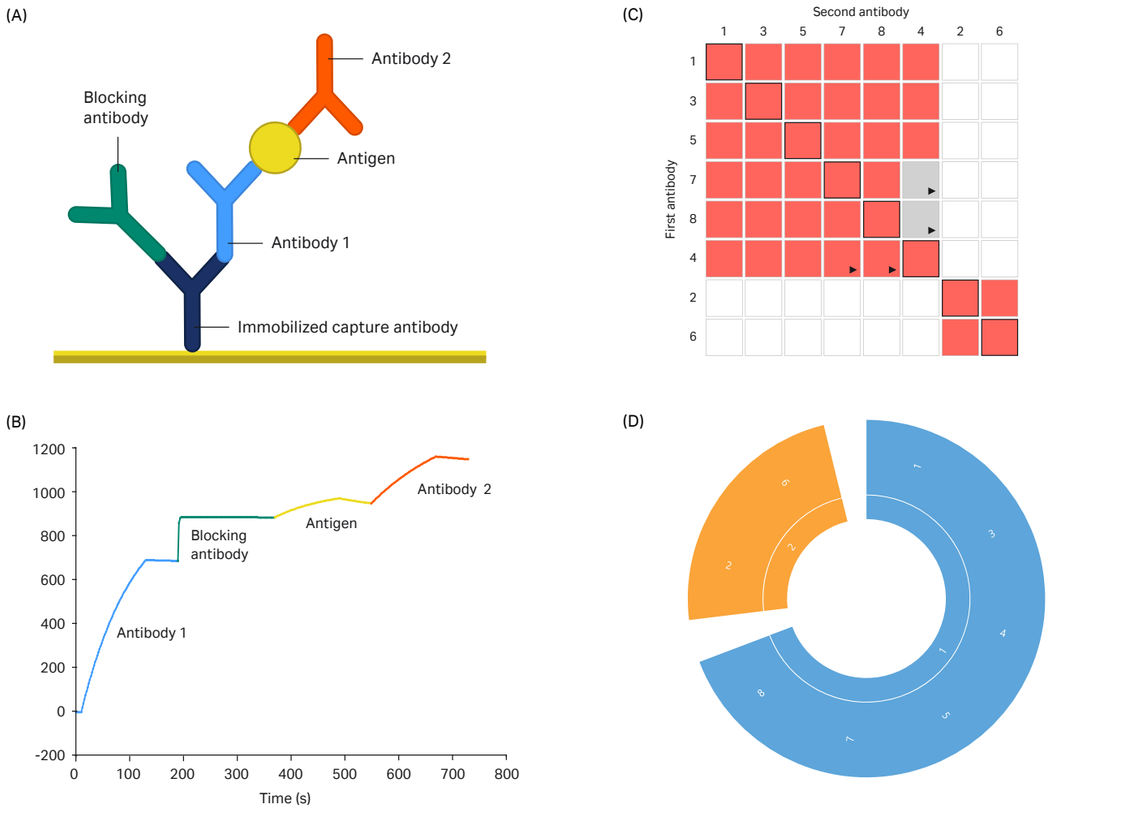


Fig 8. Epitope binning. (A) Binding sequence and molecular events, (B) sensorgram events, and (C, D) results from epitope binning experiment evaluated with Biacore™ Insight Software and Biacore™ Insight Epitope Binning Extension. The binning results from the heat map (C) is represented in a bin chart (D). Two well defined bins are identified for the eight antibodies.

Kinetic analysis for improved understanding of drug activity

For translational scientists, it is key to have the most reliable data analysis tools possible to make informed decisions. Having reliable kinetic data can help in selecting ideal lead candidates and support healthcare authority submissions — saving time and costs. Kinetic data resolves the affinity of an interaction and provides information about the rate and stability of binding. The affinity constant, K_D, is separated into an association rate constant, k_a, and a dissociation rate constant, k_d, where $K_D = k_d/k_a$

Kinetic data introduces the time domain and allows for a better understanding of target occupancy and drug residence time (28, 29). It may also contribute to the understanding of off-target effects. During humanization and re-engineering efforts, it provides detailed information on how structural changes affect binding properties. Engineering efforts may involve antigen sites, Fc-receptor binding sites for improved (30–32) or reduced (33) receptor binding, T-cell epitopes (7), introduction of linker positions for antibody drug conjugates (34), and even more complex tasks if the antibody is to be developed into a bi- or multi-specific antibody (16). Kinetics is also used in developability assays to study the effects of forced degradation on binding properties (3, 4, 22) and estimate antibody half-life using data on binding to the neonatal Fc-receptor (9). Thermodynamic analysis has also been suggested as a tool to assess developability. A screening approach to thermodynamics has been described (35) where a favorable ratio of association rate constants determined at 13°C and 37°C was indicative of the binding specificity (Fig 9).

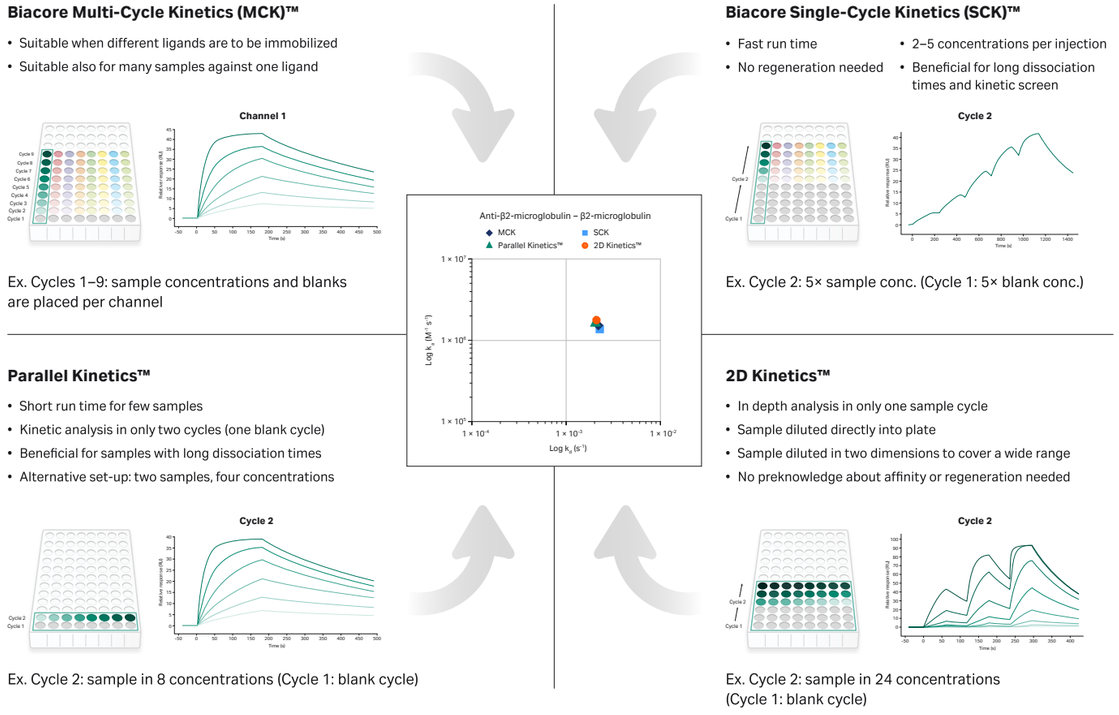


Fig 9. Depending on the preferences and circumstances of each assay, kinetic analysis can be performed in four different ways. The 2D Kinetics™ functionality offers detailed kinetics from only one sample cycle, thereby significantly reducing assay development time.

Kinetic analysis formats for rapid and informative selection of candidates

Antigens typically bind to antibodies with association rate constants in the range of 5×10^4 M^{-1} s^{-1} to 5×10^6 M^{-1} s^{-1} and with dissociation rate constants ranging from $1 \times 10^{-3} s^{-1}$ to $1 \times 10^{-5} s^{-1}$ as illustrated in the k_a vs k_d plot in Figure 10.

To illustrate possible outcomes of kinetic experiments, the rate constants associated with points A to E were used to simulate sensorgrams using varying antigen concentrations and varying injection and dissociation times. The antigen was assumed to bind antibody according to a 1:1 interaction model. The resulting scenarios illustrate different screening and characterization approaches (Fig 11).

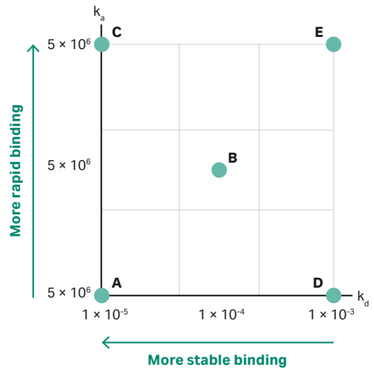


Fig 10. k_a vs k_d plot for typical antibody-antigen interactions. Response curves for conditions A to E are shown in Figure 11.

The top row shows sensorgrams based on a single injection of antigen at 10 nM concentration. Injection and dissociation times were 30 s and the maximum binding level is indicated by the red horizontal line. Binding is clearly observed for conditions B, C, and E but none of the sensorgrams reach the saturation level. The column to the right is based on normalized data and allows a direct comparison of relative dissociation rates. With a 30 s dissociation phase and low binding levels, the resolution of off-rates is poor. As the concentration of antigen is increased tenfold to 100 nM, binding associated with all conditions (A to E) is readily observed, but again the resolution of off-rates is poor. With two injections of antigen at 10 and 100 nM performed in single cycle kinetics mode, the response levels for conditions A and D are getting higher and, as the dissociation time is now 60 s, the resolution of off-rates is starting to improve. When the experiment is extended to four antigen injections and antigen is injected from 7.7 to 200 nM (3-fold dilutions) for 90 s, binding curves become more suitable for detailed kinetic analysis. Note that, with a dissociation phase of 480 s, it is easy to differentiate between k_ds of $10^{-3} s^{-1}$ and $10^{-4} s^{-1}$, and it starts to be possible to differentiate kds between $10^{-4} s^{-1}$ and $10^{-5} s^{-1}$. By extending injection and dissociation times, and increasing the concentration range, more detailed kinetic data is obtained. For practical reasons high-resolution k_d analysis will typically be restricted to a few selected samples. It will take at least 2.9 h to monitor a 10% decrease in the signal level for a dissociation rate constant of 1×10^{-5} s^{-1}. The corresponding kinetic half-life is 19.3 h.

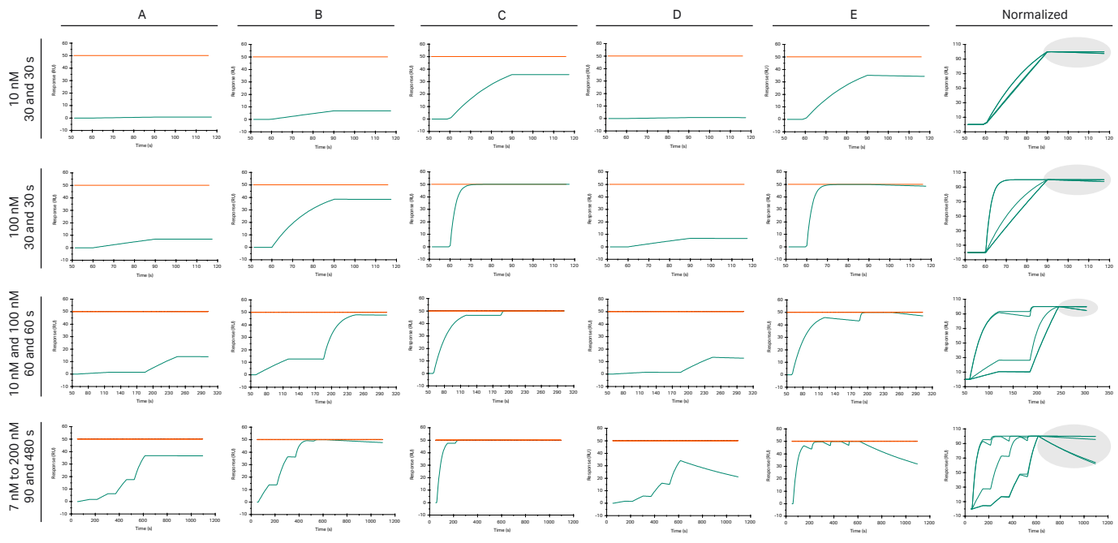


Fig 11. Antibody screening and characterization scenarios. Antigen is injected at various concentrations and for varying time as indicated to the left. Conditions A to E correspond to rate constants defined in Figure 9. The red horizontal line in A to E plots indicates the saturation response. The normalized plots were obtained by re-scaling sensorgrams on a % scale and are intended for comparison of off-rates as indicated by the red ovals.

These scenarios demonstrate how screening with one low antigen concentration can be used to select rapid binders with slow off-rates. Using injection and dissociation times of 30 s will identify slow binders and antibodies from which antigen dissociates rapidly. These antibodies can be deselected. Remaining antibodies can be re-tested using the same antigen concentration injected for a longer time (90 s) and with a longer dissociation time (10 min) to allow better differentiation of off rates. A few remaining candidates may then be characterized in more detail using a series of antigen concentrations and a further extended dissociation time.

Early indication of cell- or antibody-mediated cytotoxicity effect

Fcγ-receptors interact with IgG to regulate the immune response with implications for several disease states (36). From a therapeutic antibody perspective, binding of IgG to Fcγ-receptors may be of particular interest for anti-cancer antibodies where FcγIII-receptors on natural killer cells have been implicated in antibody-dependent cell-mediated cytotoxicity (34). IgG binds to Fcγ-receptors with varying affinity. In contrast to antibody-antigen interactions, the medium- to high-affinity interactions seen with Fcγ-receptors I and III are often complex and cannot readily be fitted to a simple interaction model (37).

The complexity most likely arises from heterogeneity in glycosylation that is found in both antibodies and receptors. Antibody binding to FcγIII-receptors is directly linked to efficacy and are important to characterize binding events. Two main approaches to Fcγ-receptor analysis have been established (38, 39). In one approach, histidine tagged Fcγ-receptor is captured by an anti-histidine antibody and antibody is injected (Fig 12A). In the second approach a Protein A surface is used. Antibody is captured and Fcγ receptor is injected (Fig 12B).

Both assay types are easy to set up, avoid covalent immobilization of antibody or receptor, and regeneration of sensor surfaces is provided as part of the capture protocols. Note that the two approaches reflect heterogeneity in different ways as the orientation of molecules differ. The shape of the sensorgrams can therefore not be expected to be identical. Kinetic analyses of these interactions are challenging due to pronounced heterogeneity (36).

Better prediction of candidate efficacy

Antibody clearance and half-life is partly determined by its interactions with the neonatal Fc-receptor, FcRn. Antibodies that are taken up into the endosome and directed for degradation in lysozomes can be salvaged from degradation and recirculated by binding to FcRn. Binding to FcRn in the endosome takes place at pH 6.0 and the antibody is released at pH 7.4 when it is returned to the cell surface (40). Increased half-life is of interest from an efficacy and dosage perspective and part of antibody engineering projects.

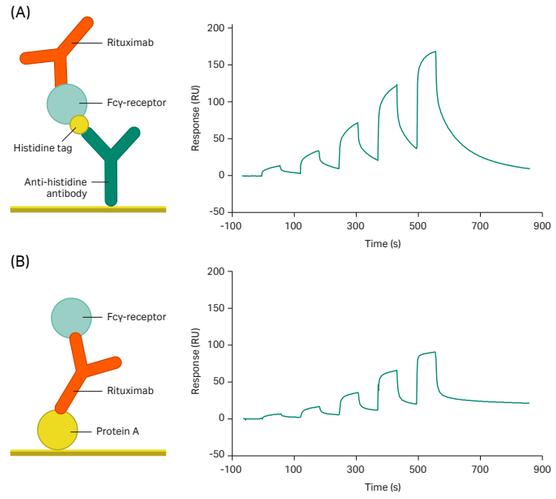


Fig 12. Antibody- Fcγ-receptor interactions are studied using either histidine tagged receptor captured on an immobilized anti-histidine antibody (A) or antibody captured to protein A (B). "A" denotes protein A and "H" denotes a histidine tag. The sensorgrams show the interaction between Rituximab and Fcγ-receptor IIIaVal 158.

The pH-dependence of antibody-FcRn interactions is easily studied in Biacore systems (Fig 13). Biotinylated FcRn is captured to streptavidin using the Biotin CAPture Kit. The running buffer is maintained at pH 6.0. Antibody is injected at pH 6.0 using Dual inject command. It binds to the receptor and when the antibody injection stops, it is immediately followed by injection of buffer at pH 7.4 to monitor dissociation at this pH. With this procedure, it is possible to seamlessly establish relevant conditions for both binding and release of antibody from FcRn. Note that the solutions for "Dual inject command" can be selected and used to introduce new buffer conditions while the running buffer remains the same.

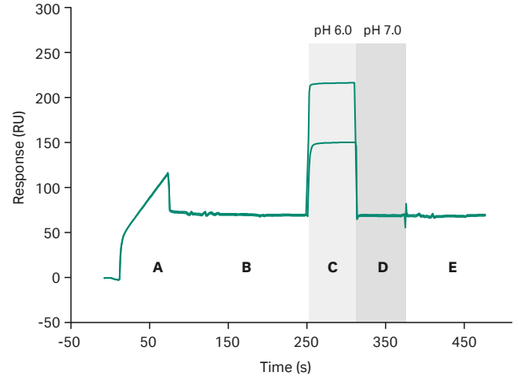


Fig 13. Binding of infliximab to hFcRn. Capture of biotinylated hFcRn (A), Running buffer PBS 6.0 (B),) ***Dual*** command phase (C and D). Infliximab injected at 333 and 2000 nM at pH 6.0 (C). Injection of pH 7.4 buffer for dissociation of infliximab (D). Return to running buffer conditions (E).

Conclusion

For translational scientists, analytical technologies that can support informed decision making can bring substantial value during the lead candidate selection process. Biacore systems deliver the resolution, sensitivity, precision, throughput, ease-of use, and assay versatility required to develop a first candidate into a clinical lead with the critical quality binding attributes required for approved biologics. Beyond selecting a lead with optimal affinity, potency, specificity, and pharmacokinetics, Biacore technology can also deliver valuable insights into numerous developability aspects, such as manufacturability and safety.

Armed with this knowledge, translational scientists can make the most informed candidate selection possible, reducing the risk of failure and costly process complications that can emerge during development. Biacore systems therefore offer a comprehensive analytical platform that enables the selection of antibodies with not only high-affinity and efficacy, but also with optimal biophysical properties to ensure a smooth journey from benchtop to clinic.

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