eBook

Face the unknown

Efficiently select and optimize antibody candidates using surface plasmon resonance

Introduction

Gaining deeper understanding of a disease pathway or discovering new therapeutics is a demanding journey. It has become more diverse as new antibodies and antibody variants emerge. Scientists leverage cutting-edge technologies to navigate the multifaceted development of these game changing therapeutics.

Selecting an antibody or antibody variant for clinical development requires a comprehensive assessment of functionality and target binding characteristics. Some targets require high affinity binding for efficient blockade or modulation, while others function optimally with lower affinity interactions.

For example, a good bispecific antibody candidate needs to have a well-balanced affinity for both targets to avoid unintended off-target binding. It must be easy to express, assemble correctly with minimal aggregation, and have high stability.

Pharmacokinetic (PK) profiles and the body´s immune response are also crucial considerations for optimal therapeutic impact and safety.

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This ebook focuses on how surface plasmon resonance and Biacore™ SPR systems are used at all stages of antibody research from selection of first candidates to clinical lead.

Label-free interaction analysis

Ligand-binding assays are key for characterization of biotherapeutic medicines. ELISA and surface plasmon resonance (SPR) are common technologies used.

The first Biacore SPR analysis system was launched 1990, and the technology is routinely used for antibody and protein characterization in cell culture, purification, and formulation workflows for the determination of antibody concentration, affinity, and kinetic analysis of drug-target interactions.

SPR measures interactions directly and can measure sequential binding events (Fig 1).

Bispecific and multispecific antibodies are a growing area of biotherapeutics. Improving assessment and understanding of multiple binding sites will be important to understanding their mechanisms of action, determining their optimal configuration, and for quality control in manufacturing. SPR measures dual-target specificities for a bispecific antibody in a single assay set up. Therefore, SPR has an important role in the ongoing development of bispecific antibodies.

Why choose SPR?

Interaction analysis can be performed for a broad range of molecular entities including small molecules, fragments, antibodies, multi-domain proteins, and viruses. SPR technology offers robust interaction measurements in various conditions, including crude matrices or weakly binding molecules. Sensor chips are reusable and the technology doesn't require labeling or reporter molecules. It can also be used with low sample and reagent volume with multiplexing capability. You can perform analyses in biologically relevant temperatures that are easily controlled with fast equilibration time.

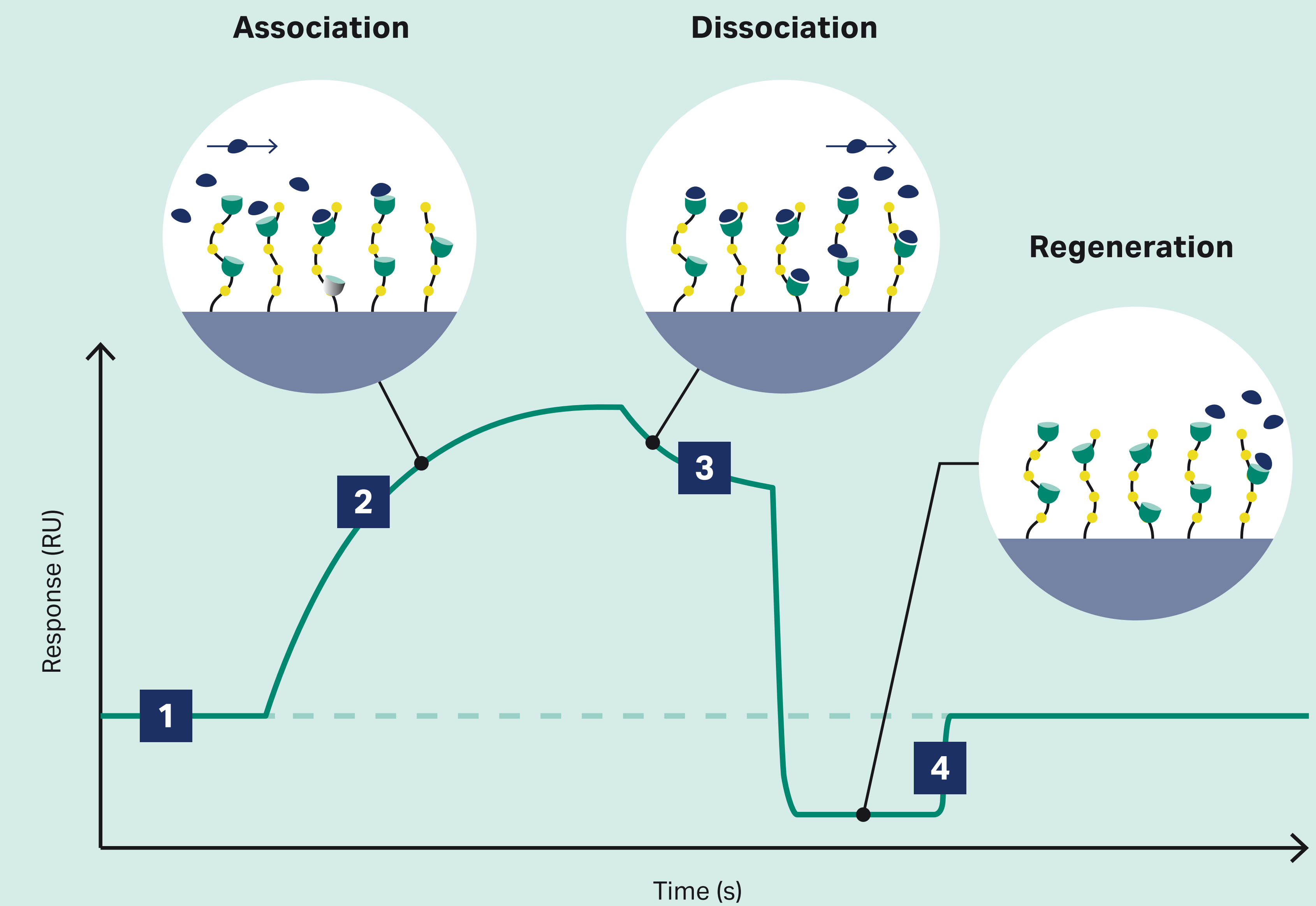


Fig 1. The molecule binding profile, displayed as a sensorgram, is used to determine affinity, kinetics, quantity, and to perform comparability studies.

Antibody and antibody variant structure

Therapeutic antibodies remain the fastest growing class of biopharmaceuticals.

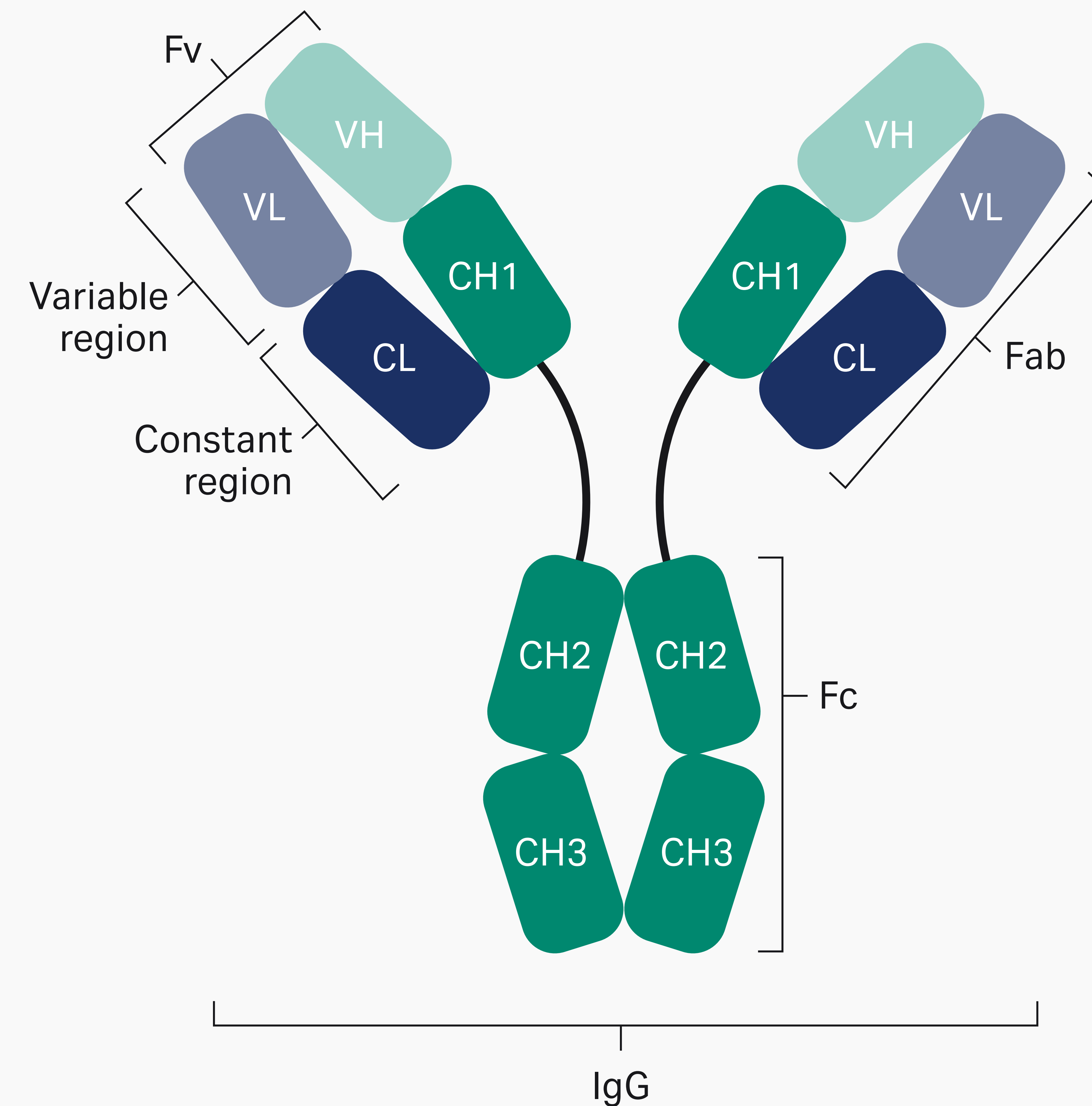
Since the FDA approved the first monoclonal based therapy in 1985, mAbs have had significant clinical successes and are used to provide effective treatment for a range of diseases. Estimated mAb world-wide sales were valued at more than $186 billion by 2022. Figure 2 shows monoclonal antibody (mAb) structure.

A bispecific antibody (bsAb) has four chains and can have various combinations of the heavy- and light-chain regions, as well as half antibodies that represent the desired bsAb and product related impurities (Fig 3). The type and number of product-related impurities depends on the assembly technique, such as Fc heterodimerization used to produce asymmetric molecules, knobs-into-holes technology, CrossMAb technology, or light-chain method used for correct pairing of light and heavy chains.

There is a huge interest in bispecific antibodies in the pharma industry. In 2023 there are only nine globally approved bispecific antibodies on the market, more than 180 bispecific antibodies in preclinical development, and another 50 in clinical trials.

Antibody structure

Fig 2. The structure of an antibody.



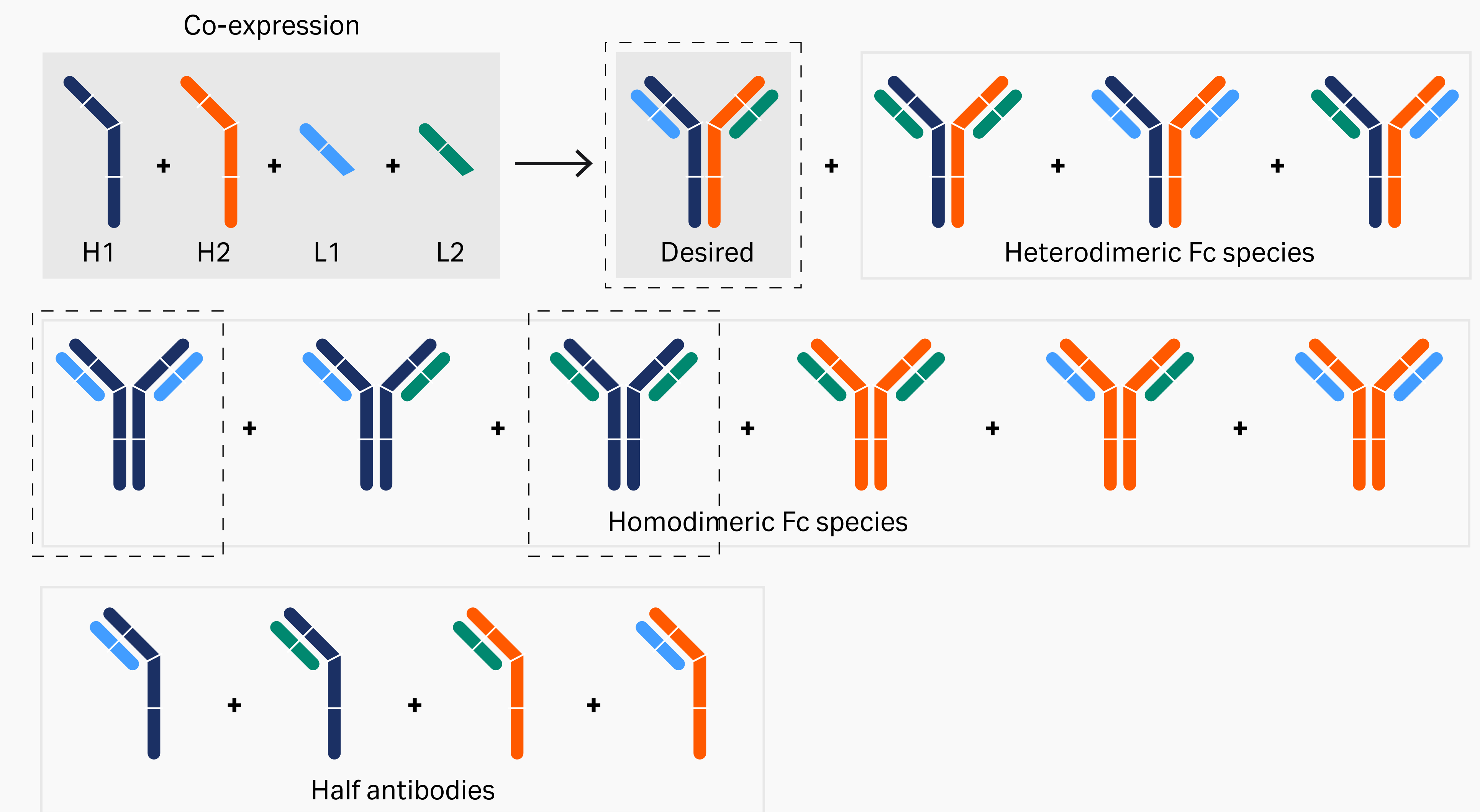


Fig 3. Possible combinations of heavy- and light-chain regions in the expression of a bsAb. The desired bispecific molecule and the mispaired homodimers are separated in the example described below.

Deeper insight into antibody characteristics — better informed hit selection

Biacore SPR technology was incorporated into antibody development workflows almost immediately after its launch in 1990 when kinetic analysis of antibody-antigen interactions and epitope binning procedures were described (1,2). Biacore systems are consistently used to determine specificity of binding to characterize antibody-antigen and antibody-Fc receptor interactions and to guide development towards a clinical lead (Fig 4). In developability studies, Biacore systems are 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 has been described for detection of changes in antibody topography because of forced degradation (3).

Efficiently select and optimize antibody candidates

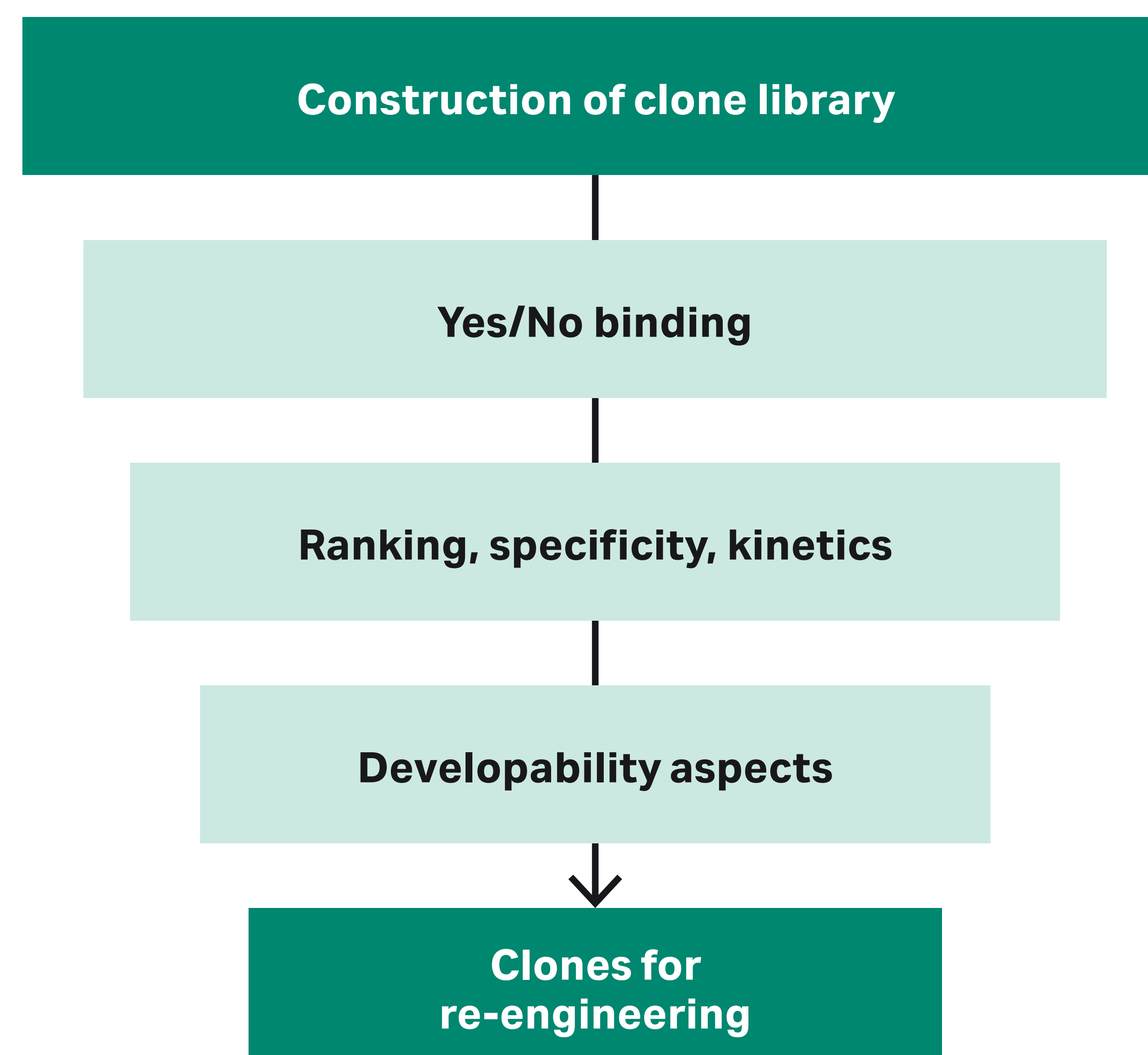


Fig 4. A stepwise approach to antibody selection. The clone library is analyzed to allow selection of candidates for re-engineering.

It has been demonstrated that several specificities in one molecule can be measured using SPR in sequence (4). However, the first binding event may result in a biased selection of antibodies that are available for the second interaction, as antibody molecules with impaired activity may not bind or bind with lower stability. It is important to determine the fraction of active molecules available for interactions.

The dual specificity of bispecific antibodies improves the chance of overcoming or slowing the progression of severe diseases such as cancer, hemophilia, diabetes, and Alzheimer's. Biacore SPR systems can be used to investigate if the binding of a bispecific antibody to antigen B is affected by the binding to antigen A.

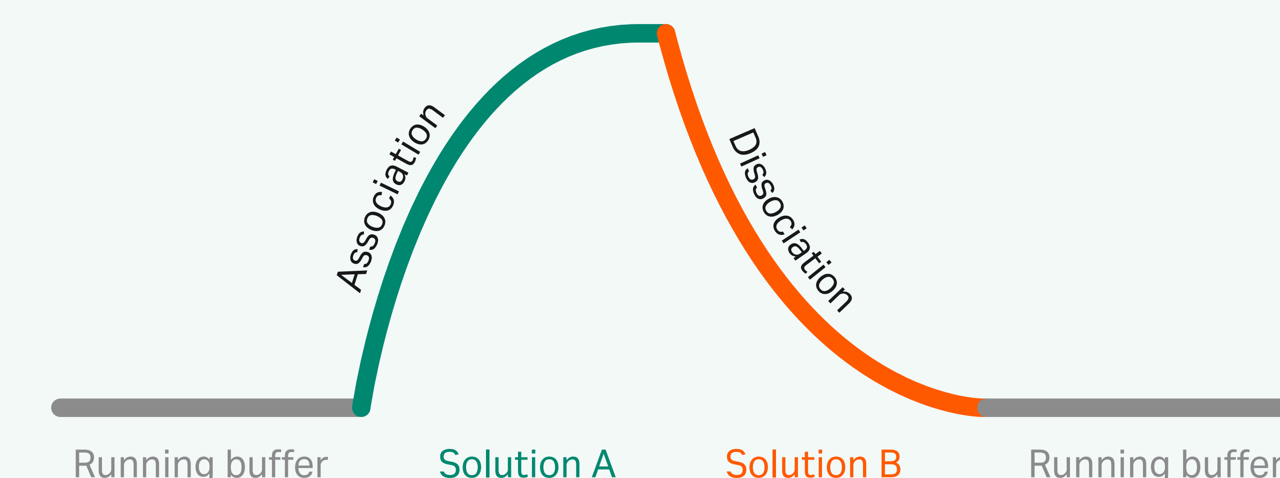
This assay was setup using the ***Dual*** command, which injects two solutions, A and B, in immediate sequence without any intermediate wash or dissociation time. Antigen B was coupled to a Biacore sensor chip. The bispecific antibody was then saturated with antigen A (solution A in Fig 5) and injected over antigen B on the surface. Once exposed to surface-bound antigen B, the bispecific antibody bound via the corresponding binding site. In the next step, a high concentration of solution A alone (solution B in Fig 5) was injected over the surface to monitor dissociation of the antibody from antigen B in the presence of antigen A to assess how the binding of antigen A affects the binding of antigen B (Fig 5).

There are more examples on how SPR can be used to measure sequential binding events that enable analysis of dual-target specificities of antibodies in a single assay set up:

- Bridge T-cell and target-cell receptors (5)
- Bridge Factor IXa and Factor (X) binding to mimic the natural function of factor FVIII (6)
- Combine VEGF and Ang-2 functionalities to reduce the formation of blood vessels (7)

Is the bispecific antibody binding to antigen A affected by the binding to antigen B?

Dual command principle



Method protocol ***Dual*** injection command

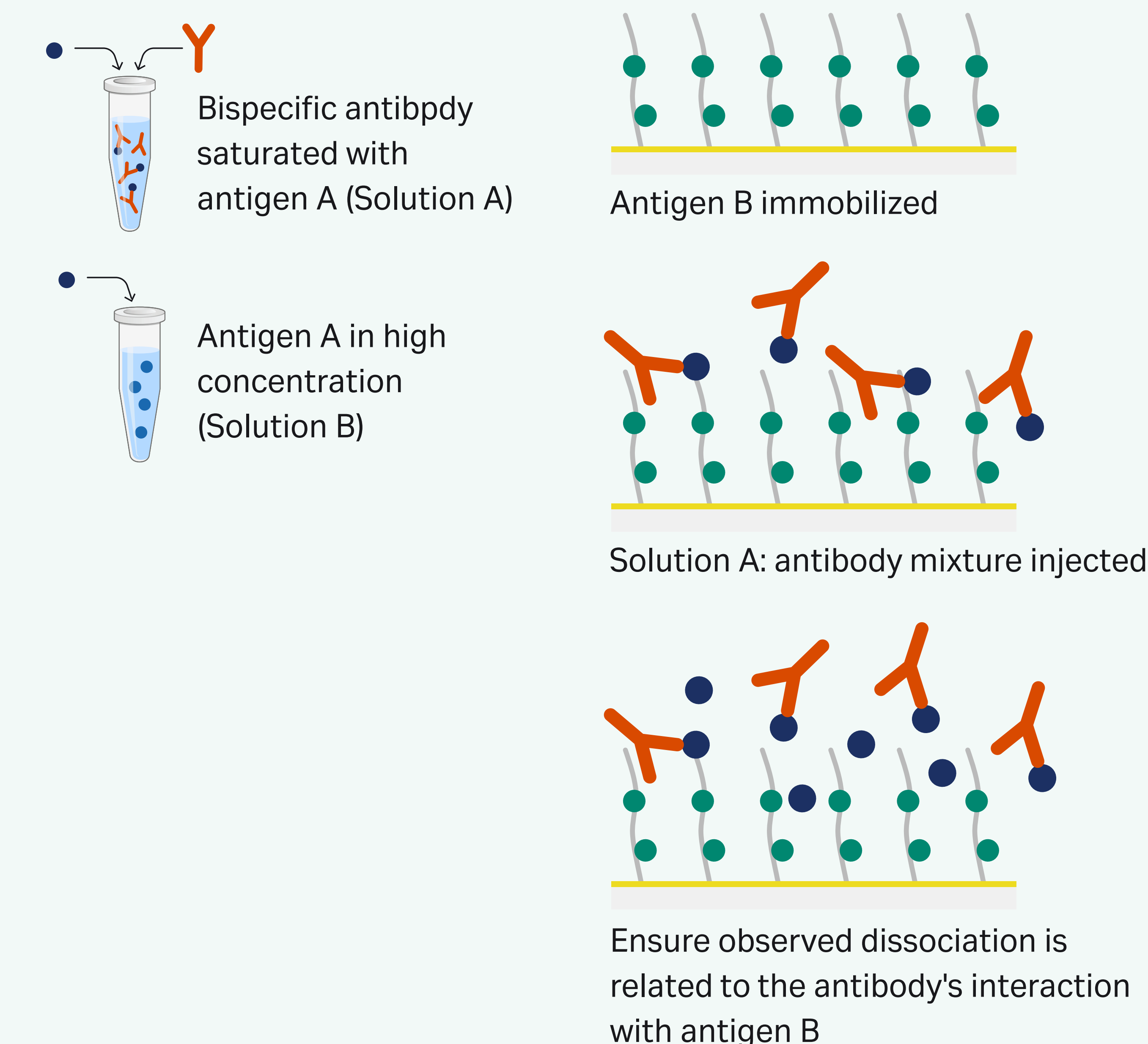


Fig 5. To determine if the bispecific antibody binding to antigen A is affected by the binding to antigen B , the assay was setup using the Dual command, which injects two solutions, A and B, in immediate sequence without any intermediate wash or dissociation time.

Early indication of cytotoxicity effects

The fragment crystallizable region (Fc region) is responsible for recognition and binding to pathogens such as parasites, bacteria, viruses, and detecting transformed cells via recognition of tumor-associated antigens.

The antibodies form an immune complex with the pathogen or antigen, which induces effector responses such as antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC) in innate immune cells through engagement with membrane-bound Fc receptors on the cell surface (8, 9).

Fc receptors recognize and interact with the Fc region of antibodies and play a critical role in immune response.

Fc receptors are glycoproteins found on the surface of effector cells such as B lymphocytes, natural killer cells, macrophages, human platelets, and mast cells. When Fc receptors interact with antigen-bound antibodies and form a membrane complex, information is communicated to the innate and adaptive immune system via tyrosine-based activation and inhibition motifs (Fig 6).

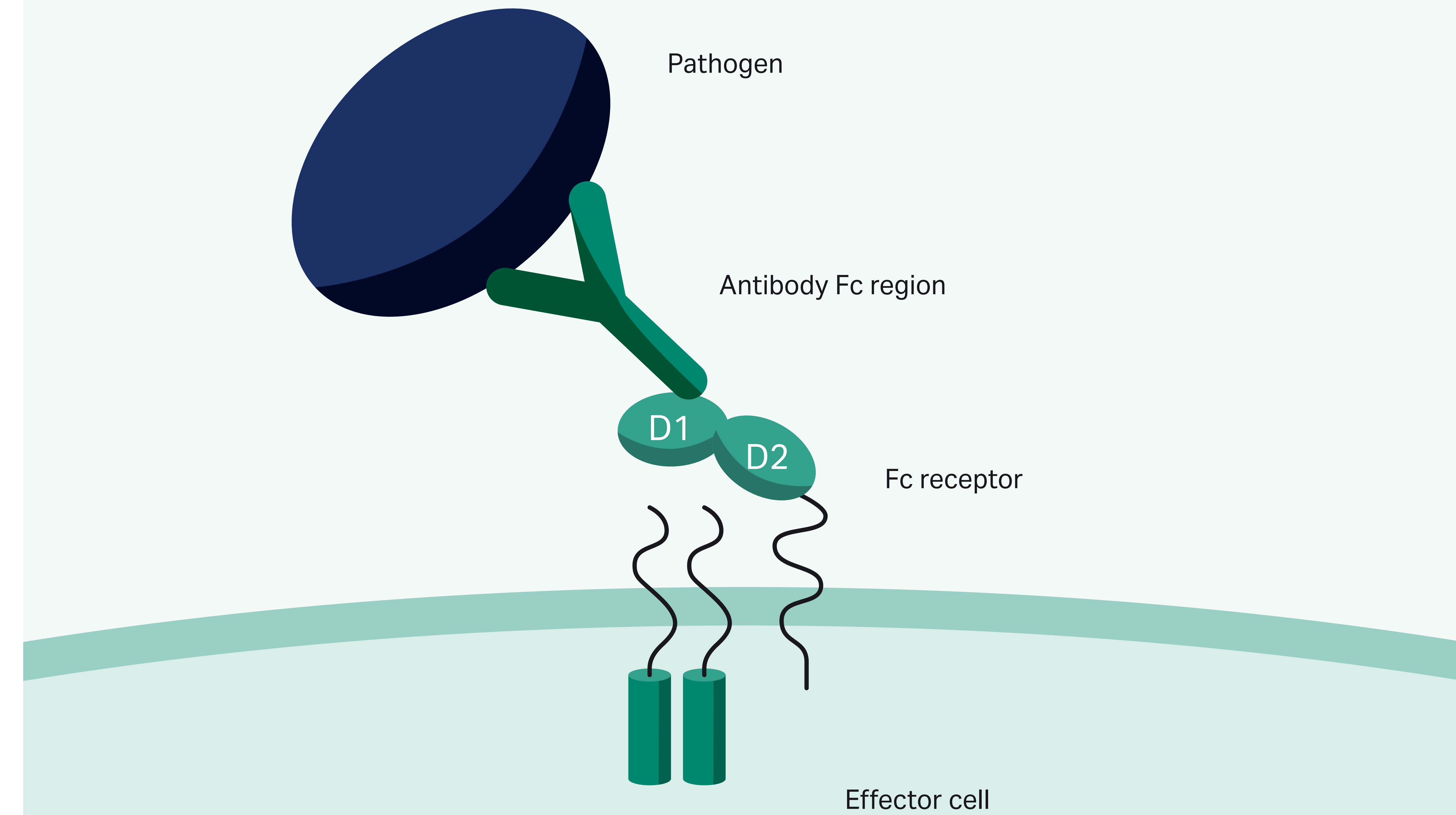


Fig 6. Interaction of Fc receptors on the cell surface of effector cells such B lymphocytes, natural killer cells, macrophages, human platelets, and mast cells with antigen-bound antibodies.

Fc-receptor binding

Tips for Fc receptor binding assays:

- Choose assay format depending on your needs and preferences.
- Choose antibody as ligand if the active concentration of your antibodies is unknown.
- Choose Fcγ receptor as ligand when there is low availability of receptors.
- Choose a capture format over direct coupling. This will result in higher repeatability and a more stable assay.
- Check for nonspecific binding from the sample matrix by injecting blank samples (sample matrix without antibody). Check for binding on both the active and reference surfaces.
- Establish that the assay is suitable for purpose using a few samples and well characterized control samples before you start extended runs with many samples.
- Prepare samples in running buffer to minimize bulk shift differences between sample and running buffer.
- When working with low concentration antibodies, care must be taken that the carryover of excipients does not cause RI effects. Spin columns or dialysis should be used for preparation of such samples.

A description on how to set up, run and evaluate Fc receptor assays on Biacore systems to establish standardized guidelines is available here.

Target-based drug discovery supported by Biacore SPR systems

Did you know that SPR was first used successfully in the characterization of the antibody-based drug, Humira, in the early 1990s. Today, SPR is the standard for real-time detection of antibody-antigen binding and kinetics in target–based drug discovery.

1990–2000

- 1990: Abbott was the first to purchase Biacore SPR system.
- Biopharma industry adopts SPR technology to determine antibody-antigen affinity, kinetics, and epitope binning.
- 1997: FDA approves first humanized mAb developed using SPR for the prevention of transplant rejection — anti-IL-2 receptor.
- Unknown molecule binding to target of interest could be identified by connecting SPR to mass spectrometry.

2001–2010

- 2003: Alefacept, the first biologic for a skin disorder, used Biacore system for drug release to ensure patient safety and drug efficacy.
- The sensitivity and throughput of SPR enables screening of large libraries of fragments or compounds.
- SPR makes binding analysis of challenging targets like G protein-coupled receptors (GPCRs) possible.

2011–2020

- First pharmaceutical developed through fragment-based drug discovery is launched.
- ~ 25% of the top 100 selling drugs-target are GPCRs.
- SPR is heavily used for secondary screening of selected HTS hits to verify elimination of false positives and promiscuous binders.
- SPR technology included in Pharmacopeia for United States, Europe, and Japan.

2021–

- Biacore SPR systems enable kinetic characterization of PROTAC ternary complex formation.
- SPR is widely used in nucleic acid vaccine development e.g., to combat COVID19.
- Biacore SPR system enables analysis of dual target specificity of bispecific antibody in a single assay.

Ready-to-go interaction analysis

We offer a range of tools designed to make Biacore SPR assays easy and reliable—backed up by stringent production methods and quality control.

Biacore Insight Software is the complete instrument control and data evaluation software for Biacore 1 series and Biacore 8 series SPR systems. Optimized solutions for common applications are offered via add-on software extensions. The software extensions provide specific functionality and tools to further streamline your analysis and reduce time to results.

Biacore sensor chips support you in the analysis of a wide range of interactions. Our functionalized sensor chips and capture kits allow you to significantly reduce assay development time, giving consistent capture of antibodies and molecules via the most common tags.

The selection guide will help you choose the best antibody attachment approach for your specific application (Fig 7).

How to choose a suitable attachment strategy for your ligand?

Antibody and antibody-like molecule

Fab fragments

Mouse Ab

Human Ab

Minimal time for assay development

Minimal time for assay development

Minimal time for assay development

Capture on kappa light chains

Capture human antibodies on kappa and lambda light chain's

Binds all mouse antibody IgG subclasses (IgG1, IgG2a, IgG2b, and IgG3), IgA, and IgM

Capture on Fc region

Specific capture in CH2 domain on FC region

Broad specificity

High capacity. Binds all IgG subclasses except IgG3

Human Fab Capture Kit

Mouse Antibody Capture Kit

Human Antibody Capture Kit

Sensor Chip Protein L

Sensor Chip CM5

Sensor Chip Protein G

Sensor Chip Protein A

Sensor Chip PrismA

First choice for immobilization on carboxyl-derivatized sensor chips

Need low immobilization level for binding kinetics

Analyte is large molecule or particle

Need for high capacity in LMW analysis. Alternative to Sensor Chip CM5

Need lower background

Interference with dextran

Sensor Chip CM5

Sensor Chip CM4

Sensor Chip CM3

Sensor Chip CM3

Sensor Chip C1

Sensor Chip PEG

Sensor Chip CM7

Fig 7. Decision tree to choose the most suitable attachment approach for antibodies and antibody variants.

Multiple ways to connect and network with subject matter experts

To learn more, download our application guides and lab procedures

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Developments in protein interaction analysis

DiPIA on-demand and as local or global scientific conferences

Collaborate, network, ask questions, and interact with subject matter experts

Biacore SPR LinkedIn community

Easy access to our calculators and SPR simulation tool

Online tools

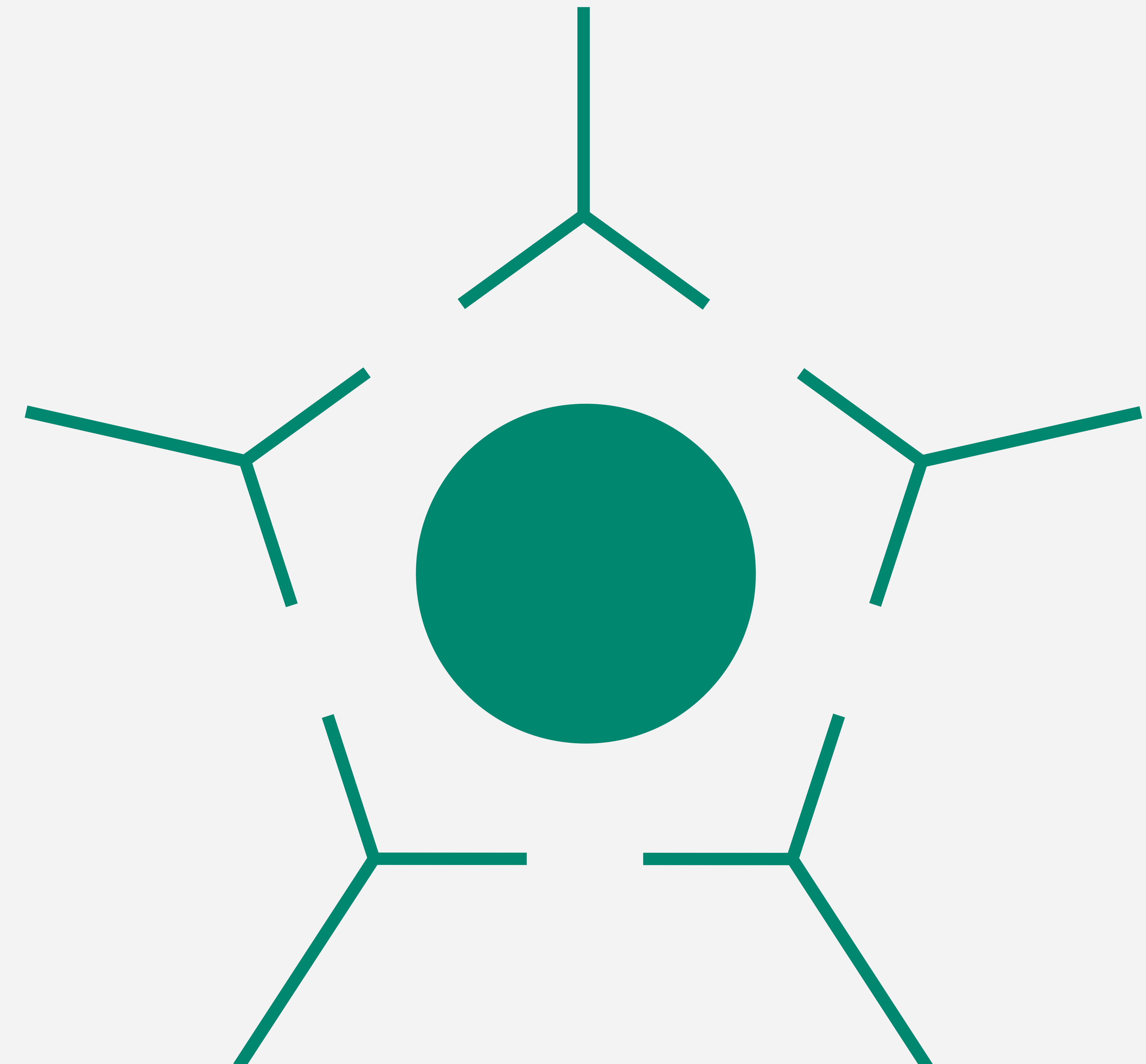
Build your own eLearning curriculum online

SPR online courses

Explore our SPR instruments in 3D

Biacore 1 series

Biacore 8 series



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