

Our **Biacore™** expertise at the service of your biotherapeutics

Affinity

Ranking

Surface Plasmon Resonance

Biacore™

Immunogenicity

ADA / NAb

Epitope Pairing

Binning / Mapping

IgG half-life

FcRn assay

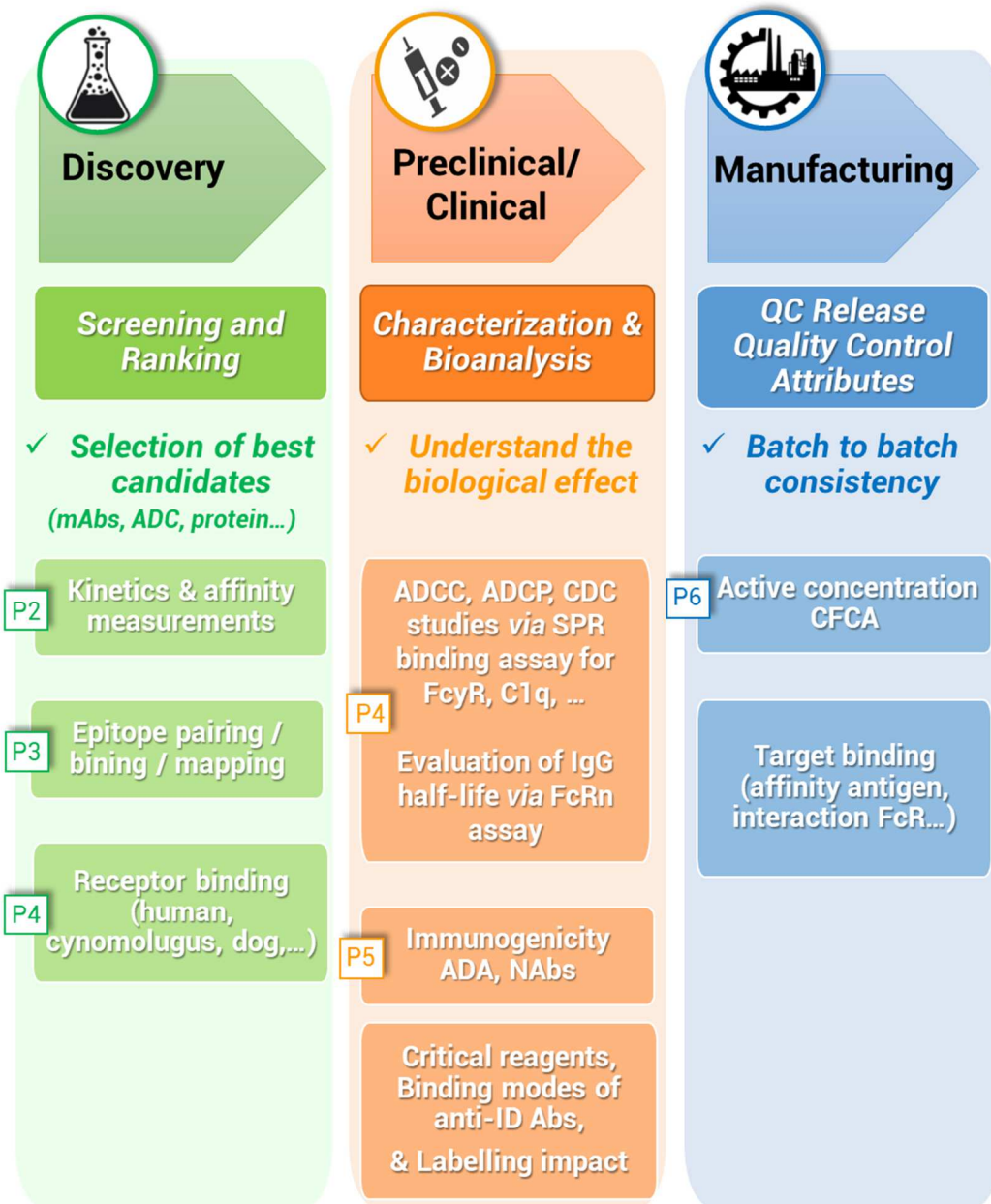
Active concentration

CFCA

Fc Receptors

ADCC / ADCP / CDC

Road Map for your Biacore™ analysis



Reglementary context: Biacore™ analysis is a response for the authority regulatory requirements

21 July 2016 EMA/CHMP/BWP/532517/2008:

Guideline on development, production, characterization and specification for monoclonal antibodies and related products.

Section 6.2 Immunological properties

Section 6.3 Biological activity

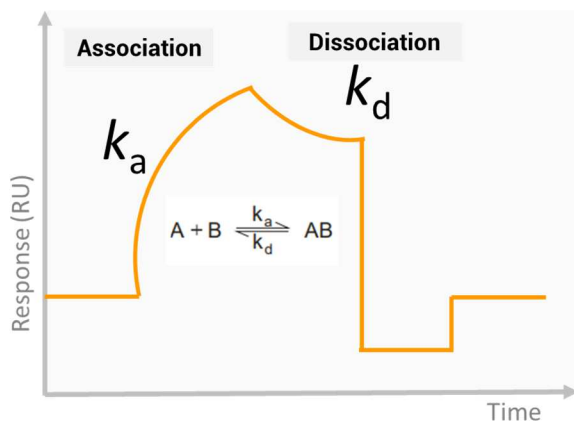
ICH Topic Q6B Specifications:

Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.

Kinetics & Affinity



Biacore™ SPR technology (Surface Plasmon Resonance) is a label-free detection method for real-time monitoring of binding interactions between two or more molecules. Biacore™ technology provides biophysical data such as kinetics and affinity.



- K_D is the **equilibrium dissociation constant**, a calculated ratio of k_d/k_a between the ligand and its analyte.
- K_D and **affinity** are inversely related, since affinity is defined as the strength of binding of the antibody to its ligand.
- Two antibodies can have the same affinity, but one may have both a high association and dissociation rates, while the other may have both low rates. It's important to focus on all **kinetics** parameters.



SELECT YOUR BEST CANDIDATES

Qualitative ranking: a rapid screening

A qualitative ranking can be performed directly on supernatant, or other matrices so that you can identify your most promising candidates.

Targets: proteins, small molecules, **ADC** (Antibody Drug Conjugates) and **biosimilars**.

- ✓ Best Affinity (K_D)
- ✓ More stable (Lowest k_d)
- ✓ Strongest (Highest k_a)



DETERMINE THE PERFORMANCE OF YOUR ANTIBODIES/MOLECULES

Quantitative kinetic characterization

Our Biacore™ service is able to accurately characterize binding kinetics and affinities of your molecules by SPR.

Proteins can be coupled either covalently or captured *via* a specific tag to a sensor surface, this allows low consumption of interacting partners.

Epitope Pairing

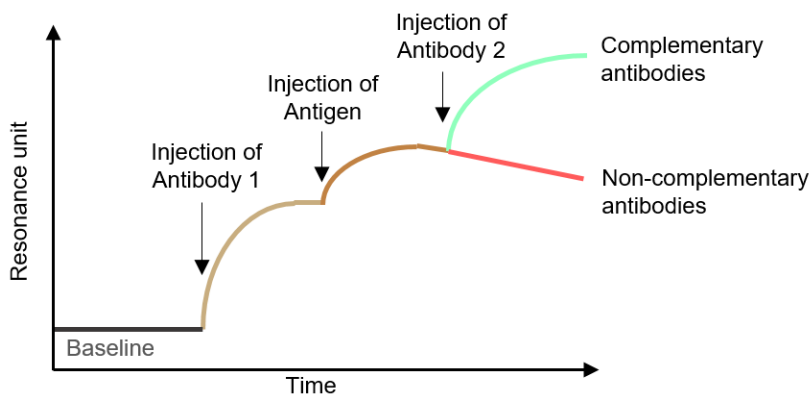


Discovery

Epitope pairing (aka epitope mapping/binning) is used to group antibodies based on the region on the antigen they are interacting with. Two antibodies recognizing the same or overlapping epitopes may compete for the binding to the antigen.

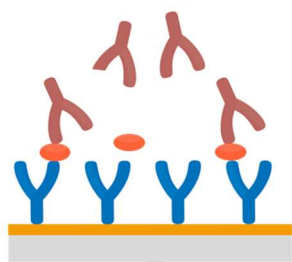


SELECT YOUR BEST ANTIBODIES FOR SANDWICH ASSAY



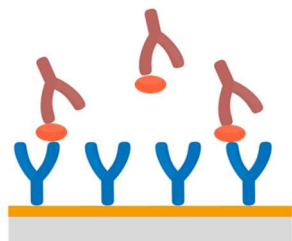
- Principle of complementarity study of human or other species IgG antibodies performed on Biacore™.

SPR allows to test the blocking effect of one antibody on the binding of a second antibody to the antigen *via* different assay formats:



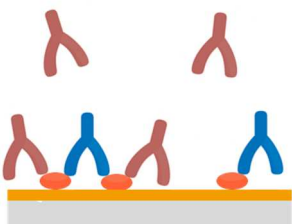
(i) Sandwich assay:

- One **immobilized antibody**
- Antigen** captured
- Injection of the **second antibody** as soluble analyte



(ii) Premix assay

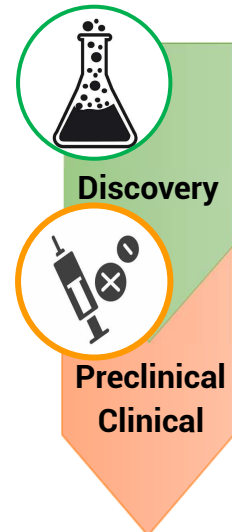
- Antigen-antibody** preincubated
- Injection of the complex on a second **immobilized antibody**



(iii) Tandem assay

- Antigen immobilized**
- Two antibodies are subsequently tested for binding to the antigen

Fc Receptors Binding Assays



By interacting with antigen at one end and cell surface Fc receptors on another, antibody effectively brings together the humoral and cell-mediated immunity. Based on their functionality and on the type of antibody they recognize, Fc-interacting proteins can be categorized into different classes.



UNDERSTAND THE FUNCTION OF YOUR Fc

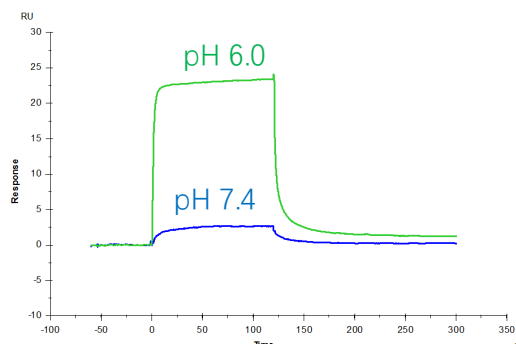
The affinity and binding specificity of the Fc domain for different FcγRs are determined by differences of the IgG subclasses (IgG1–IgG4 in humans) as well as by the structure and composition of the Fc-associated glycan structure.

Receptor	Function
FcγRI	Phagocytosis, microbe killing, cell activation
FcγRIIa	Phagocytosis, inhibition of cell activation, degranulation
FcγRIIb	Cytokine release, microbe killing, ADCC
FcγRIIc	Phagocytosis, microbe killing
FcγRIIIa	Phagocytosis, degranulation
FcγRIIIb	Transfer IgE across intestinal epithelium



UNDERSTAND THE HALF-LIFE OF YOUR IgG

FcRn	Transfer IgG across placenta, Protect IgG of degradation
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- Another class of Fc receptor is the neonatal Fc receptor for IgG (FcRn), which plays a major role in the transport of the immunoglobulin (antibody) IgG across epithelial barriers at mucosal surfaces and in the regulation of IgG half-life (recycling through rescue from lysosomal degradation).

Immunogenicity of Biologics



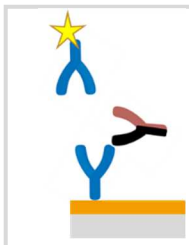
Preclinical
Clinical

Testing for immunogenicity is performed during most of the later drug development phases. According to US FDA an immunogenicity assay should be able to detect all isotypes, in particular IgM and all IgG isotypes, including IgG4. The detection and characterization of Anti-Drug Antibodies (ADAs) elicited in vivo to a biotherapeutic is a major key to assess clinical drug safety and efficacy.



DETECTION OF ADA

Powerful technique for quantitative detection of drug derived antibodies in serum samples.



IgG4 has two different specificities and can therefore not be detected by bridging ELISA

- Low affinity antibodies can be detected.
- Subtype classification of bound antibodies (IgG, IgM,...) using suitable antibodies in a subsequent sandwich experiment.



CHARACTERIZATION OF ADA

Competitive ligand-binding assays without labeling.

ADAs may induce a loss of the therapeutic efficacy of the biotherapeutics when they are neutralizing (Nabs). Positive samples in immunogenicity can be further tested for **Nabs**.

- Labeling of reagents for such assays in ELISA can potentially influenced the detection of Nabs negatively.
- Competitive ligand-binding (CLB) assay do not require labeling.

Development

Validation

Characterization

- ✓ Isotype
- ✓ Titre
- ✓ Nabs

ADA should not only be measured during drug development in pre-clinical or clinical studies, but also post-approval as a routine monitoring parameter.

Batch to Batch Consistency



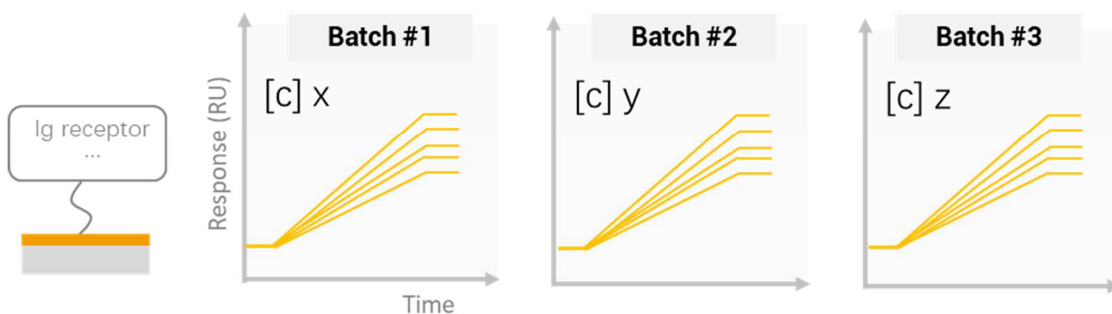
Manufacturing

For the bioproduction of Biologics, define Critical Quality Attributes (CQA) are fundamental to regulatory compliance and typically include data on, size, purity, contaminate, and how the candidate interacts with target proteins. Different Biacore™ analyses, like calibration-free concentration analysis (CFCA), are relevant to evaluate the bath to batch consistency, stability, and safety of therapeutic candidates.



DETERMINE AND COMPARE THE ACTIVE CONCENTRATION

CFCA is a new approach by Biacore™ technology **for determining the active concentration** of the molecules with respect to their interaction partners. The method is fast, reliable, requires few amount of sample and is very consistent.



- CFCA allows to compare different batches over time or different conditions.

Recommended for:

- Stability study (stress conditions: temperature, acidic condition, ...)
- Batch-to-batch consistency
- Characterization of serum antibodies

Advantage:

- No requirement of a pre-established standard curve
- Results reliable and faster

References:

Bournazos, S., Gupta, A. & Ravetch, J.V. The role of IgG Fc receptors in antibody-dependent enhancement. *Nat Rev Immunol* **20**, 633–643 (2020). <https://doi.org/10.1038/s41577-020-00410-0>

Nimmerjahn, F., Ravetch, J. Fcγ receptors as regulators of immune responses. *Nat Rev Immunol* **8**, 34–47 (2008). <https://doi.org/10.1038/nri2206>

Ben Mkaddem S, Benhamou M, Monteiro RC. Understanding Fc Receptor Involvement in Inflammatory Diseases: From Mechanisms to New Therapeutic Tools. *Front Immunol*. 2019 Apr 12;10:811. doi: 10.3389/fimmu.2019.00811. PMID: 31057544; PMCID: PMC6481281.

Bournazos S, Wang TT, Dahan R, Maamary J, Ravetch JV. Signaling by Antibodies: Recent Progress. *Annu Rev Immunol*. 2017 Apr 26;35:285-311. doi: 10.1146/annurev-immunol-051116-052433. PMID: 28446061; PMCID: PMC5613280.