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Principles of Immunogenicity Assessment Using Biacore™ T200 SPR System

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Immunogenicity Assessment

ADAPTIVE IMMUNITY

INFLAMMATION AND INNATE IMMUNITY

CELL-MEDIATED

ANTIBODY-MEDIATED

CELLULAR COMPONENT

HUMORAL COMPONENT

T-dependent antigens (T-D)

T-independent antigens (TI-1 and TI-2)

Antibody-Mediated Immune Response

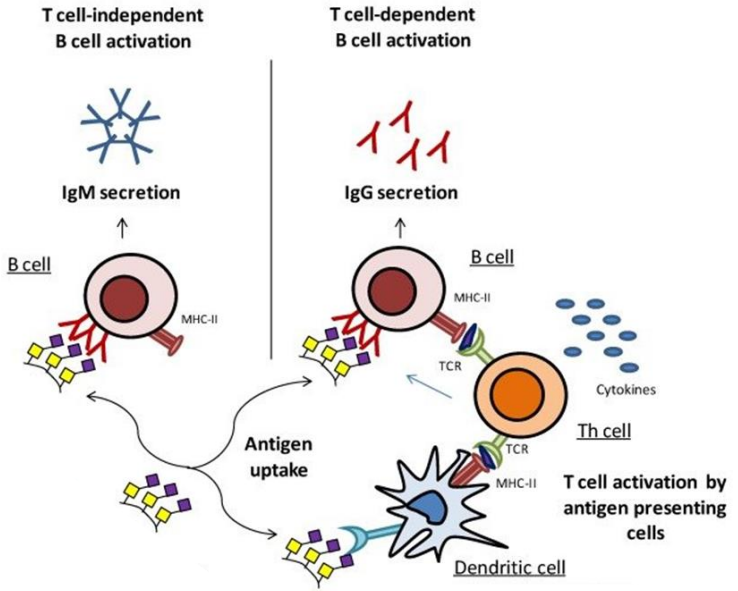
Two categories of antigens regarding their recognition and the induction of humoral immune response:

T-dependent (T-D) antigens:

- Endocytosed by antigen presenting cells (APCs)
- Presentation to T_H cells
- T cell activation
- T cell-dependent B cell activation and IgG secretion

T-independent (T-I) antigens:

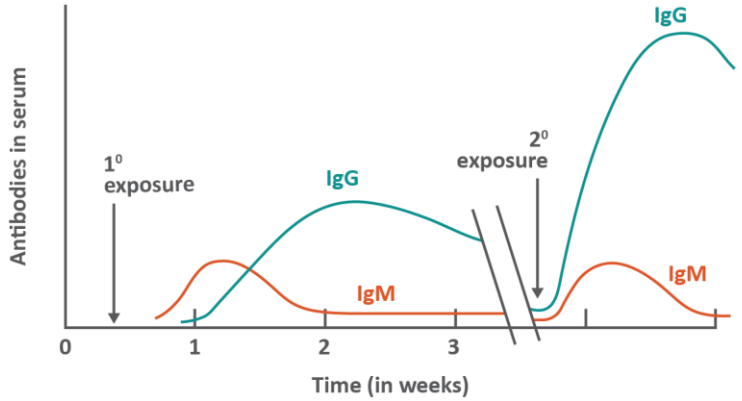
- Directly recognized by B cells
- Cross-linking of the B cell receptors
- T cell-independent B cell activation and IgM secretion



Sylvain et al., (2012). Biomolecules. 2. 435-466

Immunogenicity of Biologics

- Therapeutic proteins can **induce an immune response (anti-drug antibodies).**
- Effects range from **no clinical effect to serious adverse effects.**
- Immunogenicity testing is **essential to ensure:**
  - **Clinical safety & efficacy**
  - **Regulatory compliance**



Causes of Immunogenicity

Structural Properties

- Sequence variation
- PTM/Glycosylation
- Aggregation, oxidation, degradation, deamination
- Conformational changes

Treatment Related

- Dose
- Route of application
- Frequency of application
- Length of treatment

Immunogenicity

Manufacturing Process

- Contaminants/impurities
- Production/purification
- Storage conditions
- Formulation

Patient & Disease Related

- Immune status
- Genetic background
- Concomitant treatment
- Pre-existing antibodies

Consequences of Immunogenicity

No Clinical Effect

Altered PK/PD Profile

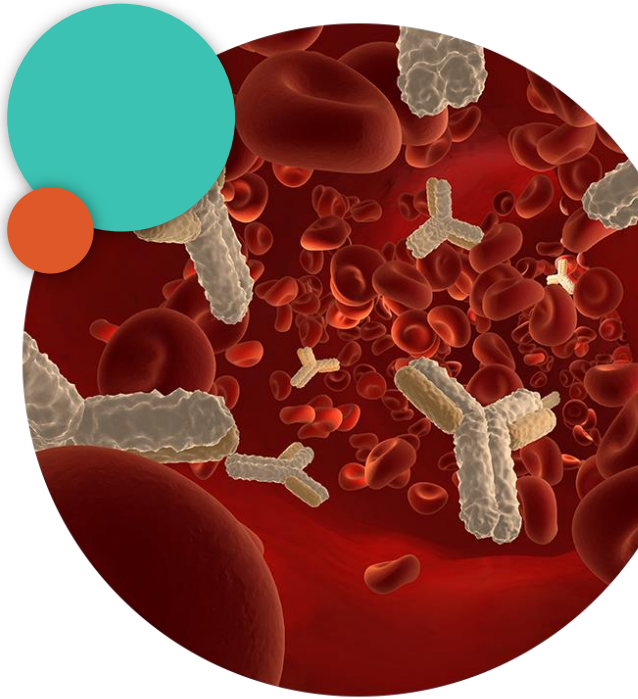
- Increased/decreased drug exposure

Hypersensitivity

- Anaphylactic/ anaphylactoid reactions

Neutralizing Antibodies

- Reduced drug efficacy
- Neutralization of endogeneous counterpart



Examples of Serious ADA Effects

Biotherapeutic	Indication	Consequences
rDNA Human MGDF (Pegylated)	Increase platelets during Chemotherapy	• MDGF induces Abs neutralizes the TPO leading to auto-immune Thrombocytopenia • Cross reacted with endogenous protein and caused adverse effects.
Erythropoietin (EPO)	**Anemia**	• **NAb to EPO induces PRCA (pure red-cell aplasia)** • **Cause formulation change (particulate) and route of administration** • **Cross reacted with endogenous protein and caused adverse effects.**
Glucocerebrosidase (Placental derived)	Gaucher patients	• ~13% patients developed Abs (1/3rd NAb cases) • 90% of these patients become tolerized over time • Loss in efficacy
Factor VIII	Hemophilia	• Up to 35% patients develop Abs • Loss in efficacy
Recombinant human Insulin	Diabetes mellitus	• Up to 44% of patients, IgE Abs in ~5% patients with insulin allergy • Note: Lipoatrophy with nonpurified bovine/porcine insulin

Regulatory Requirements FDA (2019)

"Screening assays […] are used to detect antibodies that bind to the therapeutic protein product. […] the screening assay should be sensitive and designed to detect low levels of **low- and high-affinity** ADA […]."

"The **specificity** of ADA for the therapeutic protein product is usually established by competition with a therapeutic protein in a confirmatory assay."

"Titration assays characterize the **magnitude of the ADA response**. It is important to characterize this magnitude with titration assays because the impact of ADA on pharmacokinetics, pharmacodynamics, safety, and efficacy may correlate with ADA titer and persistence rather than incidence (Cohen and Rivera 2010)."

"Neutralization assays assess ADA for **neutralizing activity**. It is important to characterize neutralizing activity of ADA because the impact of ADA on pharmacokinetics, pharmacodynamics, safety, and efficacy may correlate with NAb activity rather than ADA incidence (Calabresi et al. 2007; Goodin et al. 2007; Cohen and Rivera 2010; Wang et al. 2016; Wu et al. 2016)"

"For **non-mucosal** routes of administration and in the **absence of a risk of anaphylaxis**, the relevant ADA isotypes are **IgM** and **IgG**."

"For **mucosal** routes of administration, **IgA** isotype ADAs are also relevant."

"[…] for therapeutic protein products where there is a **high risk for anaphylaxis** or where **anaphylaxis has been observed**, results from antigen-specific **IgE** assays may be informative."

"[…] generation of **IgG4** antibodies has been associated with immune responses generated under conditions of **chronic antigen exposure**, such as factor VIII treatment, and in erythropoietin-treated subjects with pure red cell aplasia (Matsumoto et al. 2001; Aalberse and Schuurman 2002)."

Tiered Approach for **Antibody Response Assessment**

ADA Assay Validation

Cut Point(s)	Statistical threshold to distinguish positive/negative samples
Sensitivity & Drug Tolerance	Limit of detection (~100 ng/mL); in presence of therapeutic protein at trough levels
Specificity & Selectivity	Exclusively detect the target analyte; in presence of other sample components
Precision	Variability within and between assay runs
Robustness & Stability	Variations in method and instrument performance

Biacore™ T200 SPR Platform

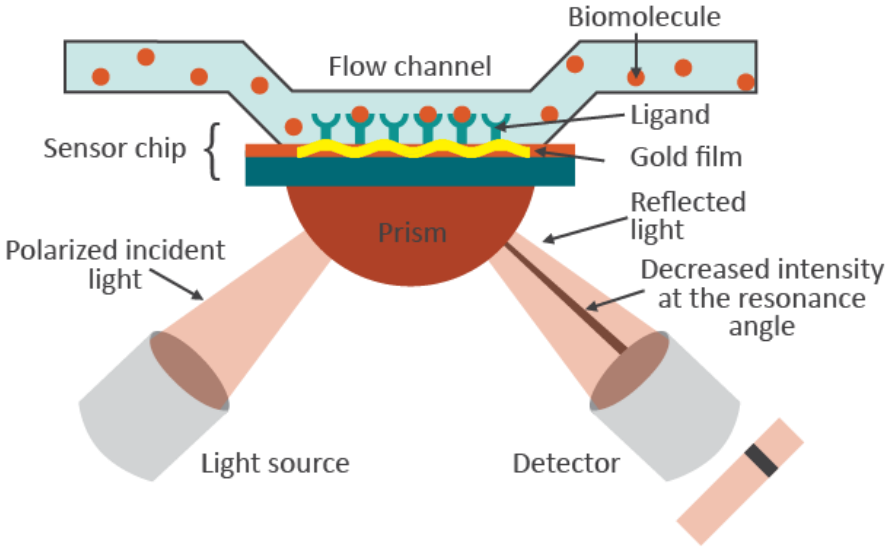
Biacore™ T200

- **Analyzes and characterizes ADAs and molecular interactions** related to kinetics, specificity, and concentration.
- **Is a non-invasive label-free** technology based on surface plasmon resonance (SPR) principle.
- **Reacts to changes in the concentration of molecules** at the sensor surface as molecules bind to or detach from the surface.

Biacore™ T200 SPR Platform

What is surface plasmon resonance (SPR)?

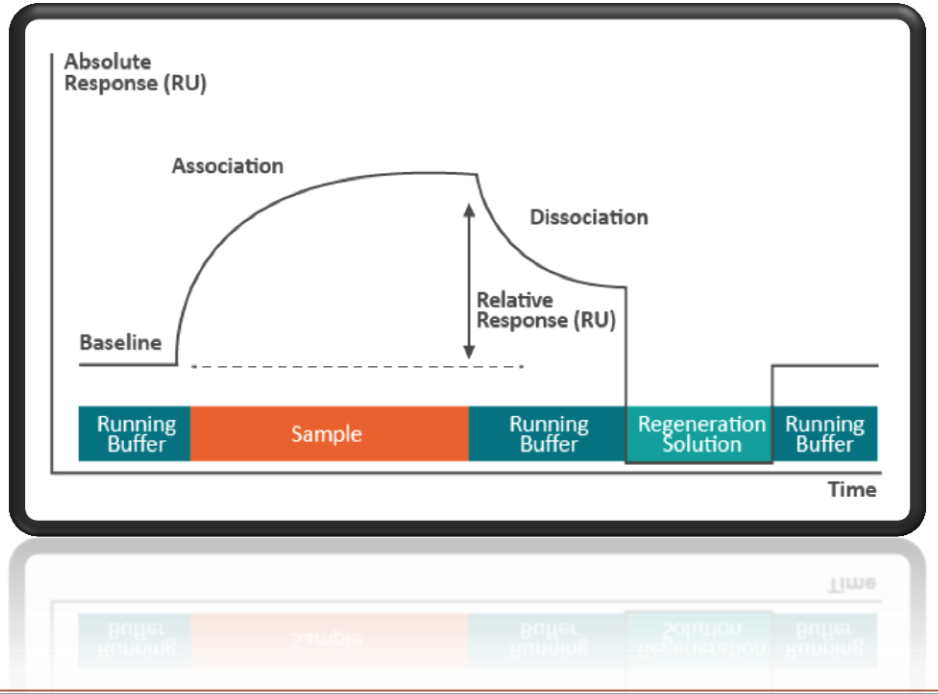
- SPR allows **real-time, label-free detection of biomolecular interactions.**
- SPR occurs when **polarized light** strikes an electrically conducting surface at the interface between two media.
- This generates **electron charge density waves called plasmons**, reducing the intensity of reflected light at a specific angle known as the **resonance angle**, in proportion to the mass on a sensor surface.



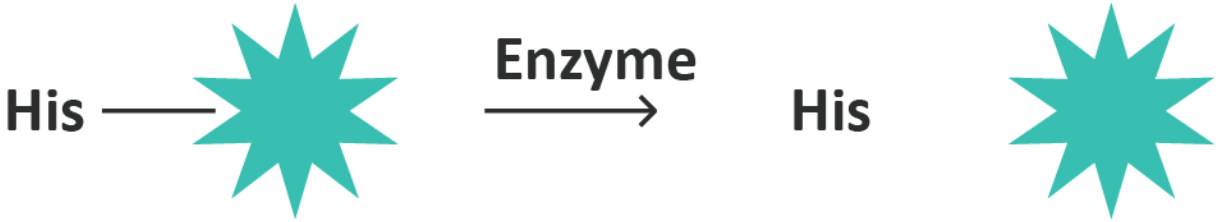
Biacore™ T200 SPR Platform

What data can be obtained from an interaction?

- **Binding:** Does the interacting partner bind to the target molecule?
- **Specificity:** To what extent does an interacting partner cross-react with other molecules?
- **Concentration:** How much of a given molecule is present and active?
- **Kinetics:** What are the rates of association and dissociation?
- **Affinity:** How strong is the binding?



ADA – Screening Assay

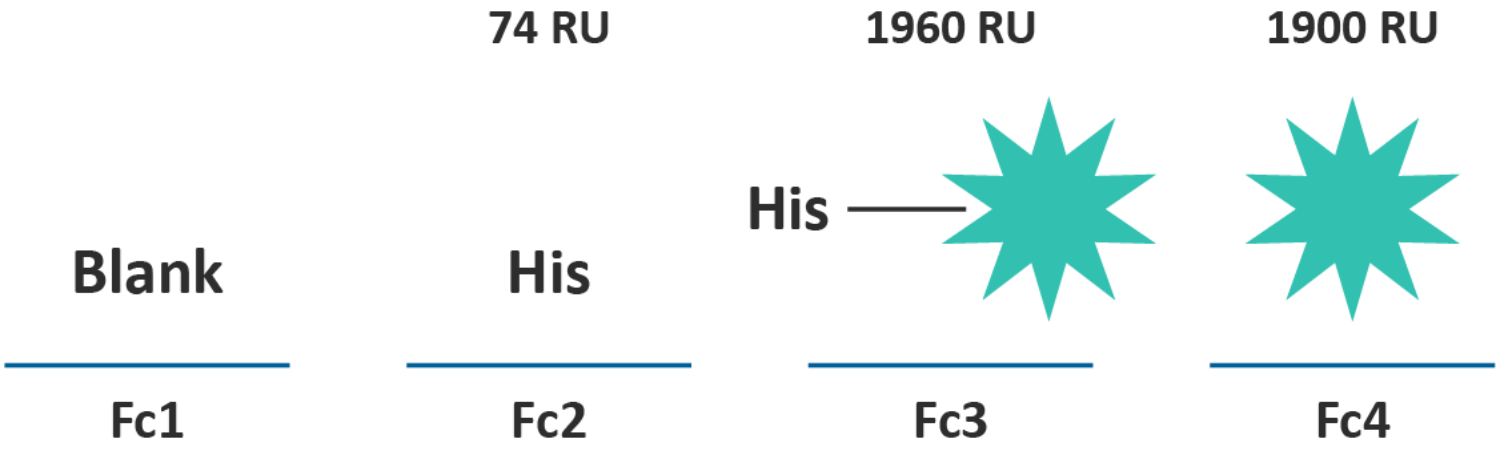


His-tag enzymatically removed after purification.

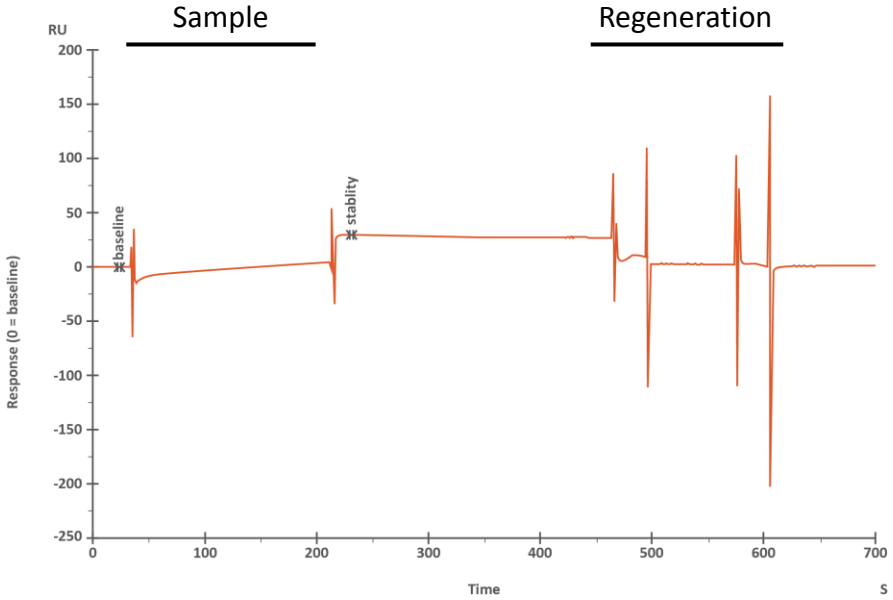
Immunogenic byproducts/residuals?

If ADAs are formed, authorities may ask against which target.

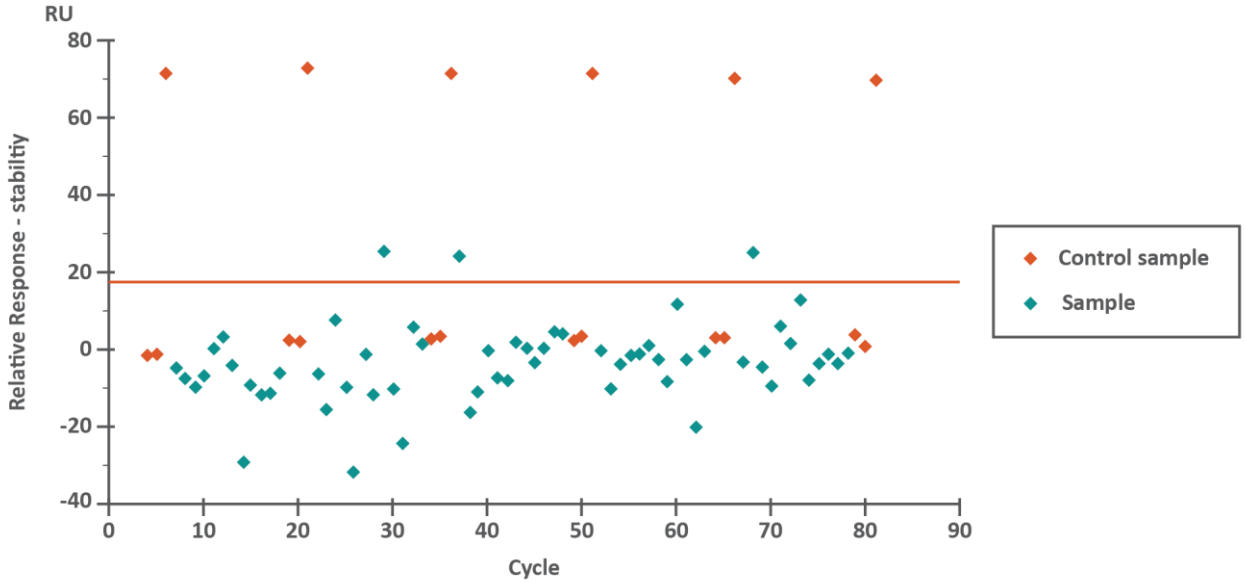
ADA – Screening Assay



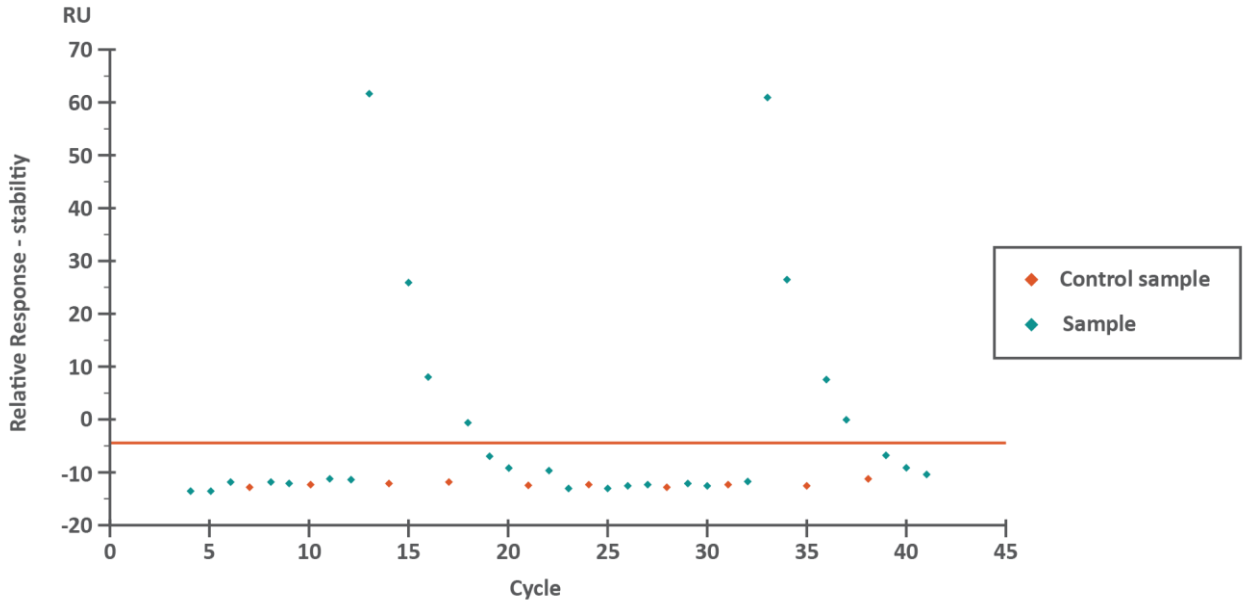
ADA – Screening Assay



ADA – Screening Assay – Cut Point



ADA – Screening Assay – Sensitivity



ADA – Screening Assay – Qualification

Regeneration Test	Surface Performance/ Stability Test	Minimum Required Dilution
3 Cut Points	Sensitivity: 1 µg/mL	Precision: 2.8 %CV Intra-Assay 3.6 %CV Inter-Assay

ADA – Screening Assay

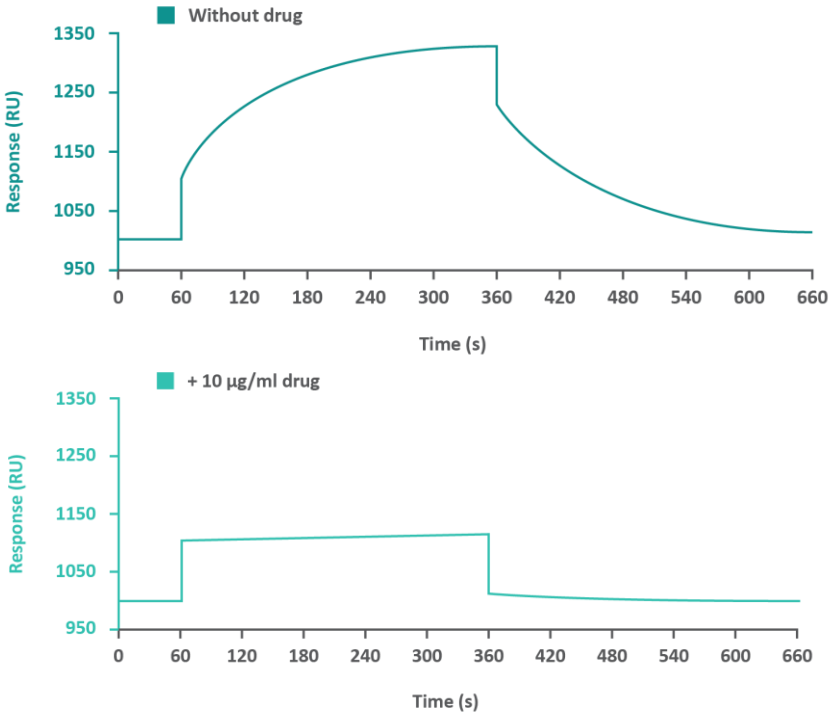
Lower sensitivity compared to ELISA, but allows detection of low-affinity ADAs.

Drug	No. of Positives (ELISA)	No. of Positives (SPR)
Iodine 131 chimeric tumor necrosis mAb[1]	4/78	7/78
Biotherapeutic drug, Merck Serono[2]	19/62	25/62
Panitumumab[3]	2/612	25/604
rhEPO[4]	6/8	8/8

[1] Wang *et al.* Cancer Immuniol. Immunother. 57 (2008)
[2] Presented at Immunogenicity for Biologics, Munich (2011)
[3] Lofgren *et al.* J. Immunol. 178 (2007)
[4] Swanson *et al.* Clin. Pract. 96 (2004)

ADA – Confirmatory Assay

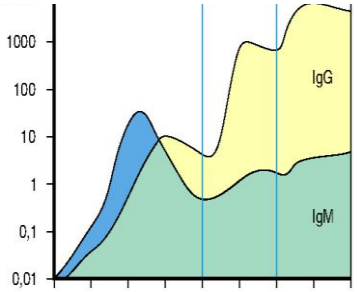
- **Drug depletion assay**
- Inhibition of response by adding **excess of drug to the sample**
- **Confirms that response derives** from specific binding to the drug



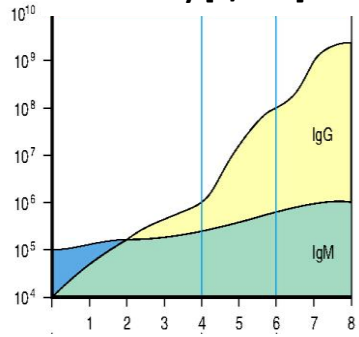
Clinical Relevance of Antibody Isotypes

	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ1	γ2	γ3	γ4	μ	α1	α2	δ	ε
Molecular weight (kD)	146	146	165	146	970	160	160	184	188
Concentration [mg/ml]	9	3	1	0,5	1,5	3,0	0,5	0,03	5×10^{-5}
Half life time [days]	21	20	7	21	10	6	6	3	2
Complement activation	++	+	+++	−	+++	−	−	−	−
Alternative pathway	−	−	−	−	−	+	−	−	−
Placenta transfer	+++	+	++	−/+	−	−	−	−	−
Binding to MΦ	+	−	+	−/+	−	+	+	−	+
Binding to mast cells	−	−	−	−	−	−	−	−	+++
Reactivity to Protein A	+	+	−/+	+	−	−	−	−	−

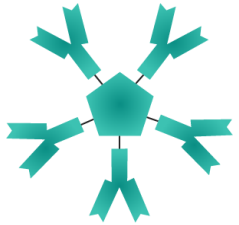
Concentration [µg/ml]



Affinity [1/mol]

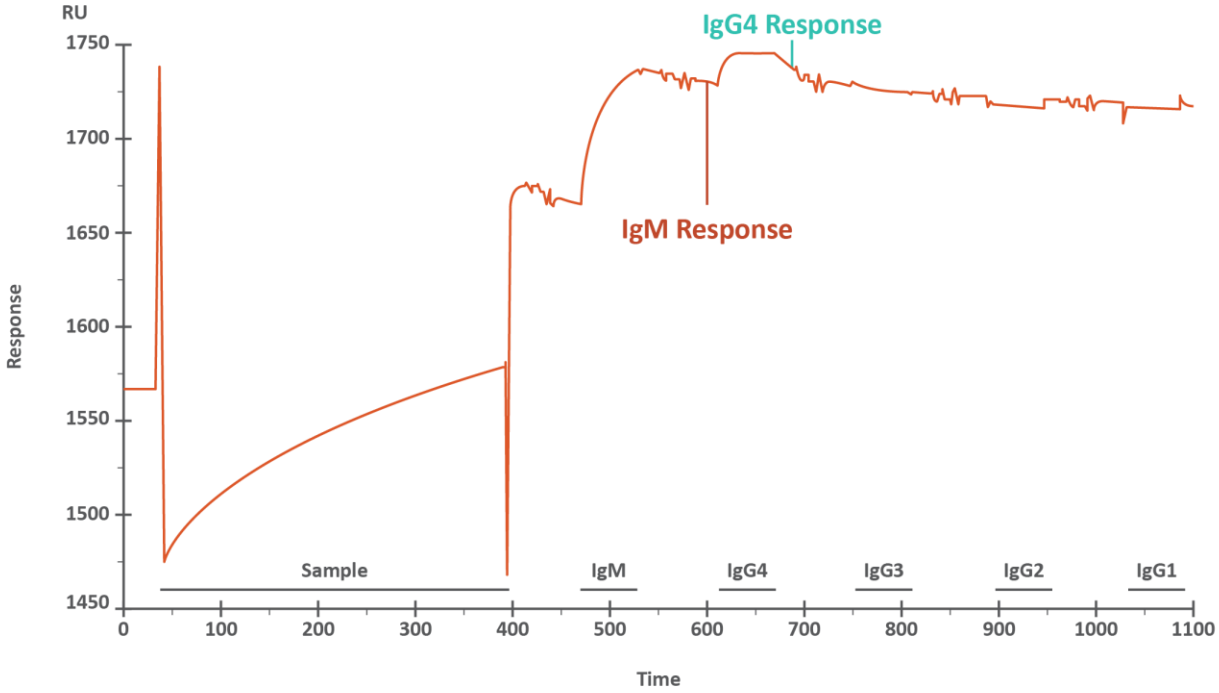


IgM



IgA / IgD / IgE / IgG

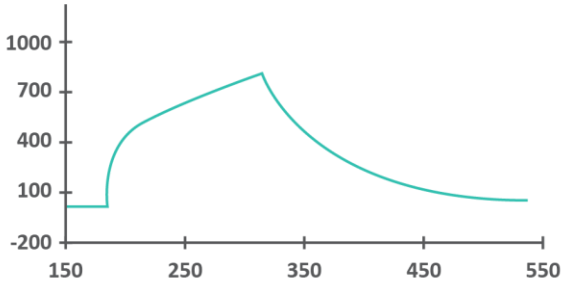
ADA Isotyping

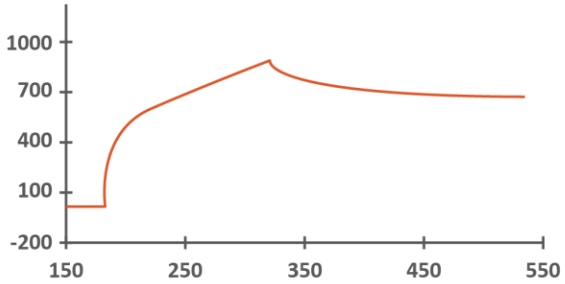


ADA Isotyping – Binding Stability

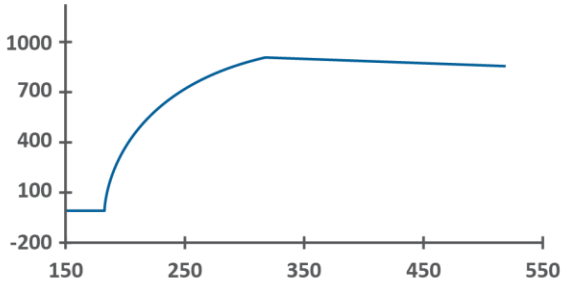
Assessment of binding stability enables monitoring of ADA maturation.

Rapid dissociation
Weak interaction



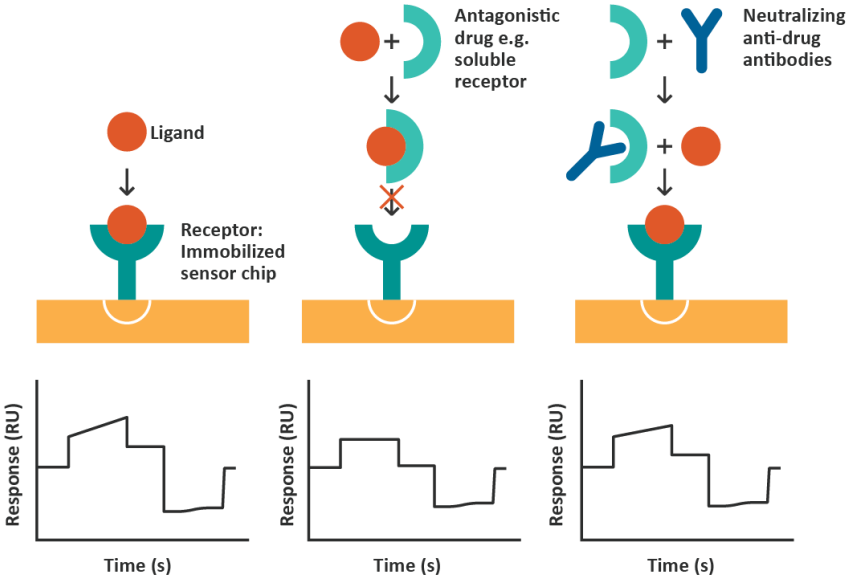


Slow dissociation
Stable interaction



Neutralizing Antibodies

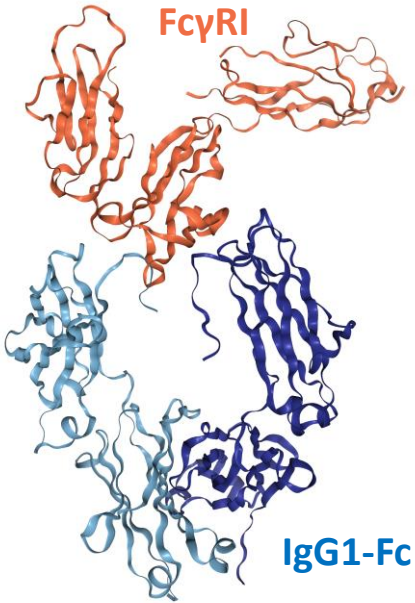
- **Competitive ligand binding (CLB) assay** → for antagonistic drugs against humoral targets.
- Needs to **reflect Mechanism of Action.**
- **Drug binds to soluble ligand,** thereby preventing it from binding to receptor.
- **Presence of NAbs inhibit the antagonistic effect of the drug.**



Functional testing of TMAB

Biological Effector Function

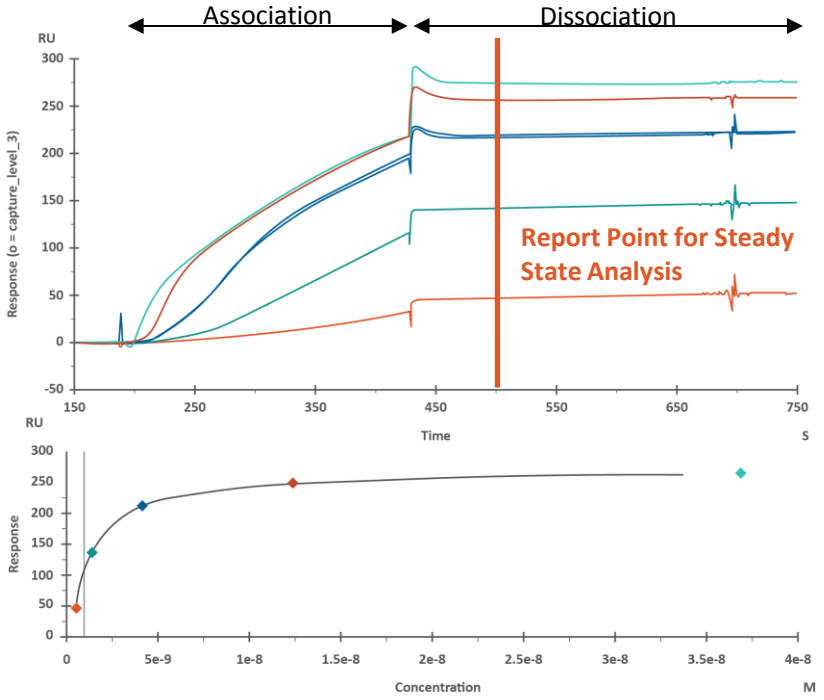
Receptor	Principal Ligand	Affinity	Cell Distribution	Function
FcγRI (CD64)	IgG1; IgG3	High (nM)	Macrophages, neutrophils, eosinophils, dendritic cells	Phagocytosis, cell activation, respiratory burst, microbe killing
FcγRII (CD32)	IgG	Med (nM-μM)	Macrophages, neutrophils, eosinophils, platelets, B cells, mast cells	Phagocytosis, degranulation, inhibition of cell activity
FcγRIII (CD16)	IgG	Low (μM)	NK cells, macrophages, neutrophils, eosinophils, mast cells	Cytokine release, microbe killing, ADCC
FcαRI (CD89)	IgA	Low (μM)	Monocytes, macrophages, neutrophils, eosinophils	Phagocytosis, microbe killing
FcεRI	IgE	High (pM)	Monocyes, mast cells, eosinophils, basophils	Phagocytosis, degranulation
FcεRII (CD23)	IgE	Med (nM-μM)	B cells, eosinophils	Transport IgE across intestinal epithelium, enhance allergic sensitization
FcRn	IgG	High (nM)	Monocytes, macrophages, hepatocytes, dendritic, epithelial and endothelial cells	Transfer IgG across placenta; protects IgG from degradation



Lu *et al.* Proc. Natl. Acad. Sci. 112 (2015); PDB ID: 4X4M

Effector Function

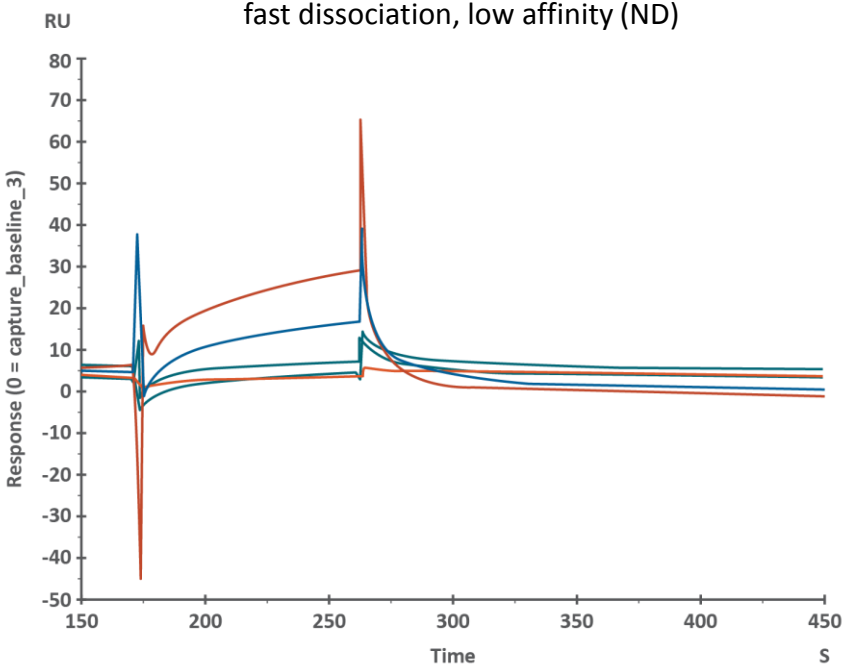
- Modified antibody modality binding to FcRn
- Stable binder; affinity determined from steady state
- K_D: ~1 nM



Effector Function

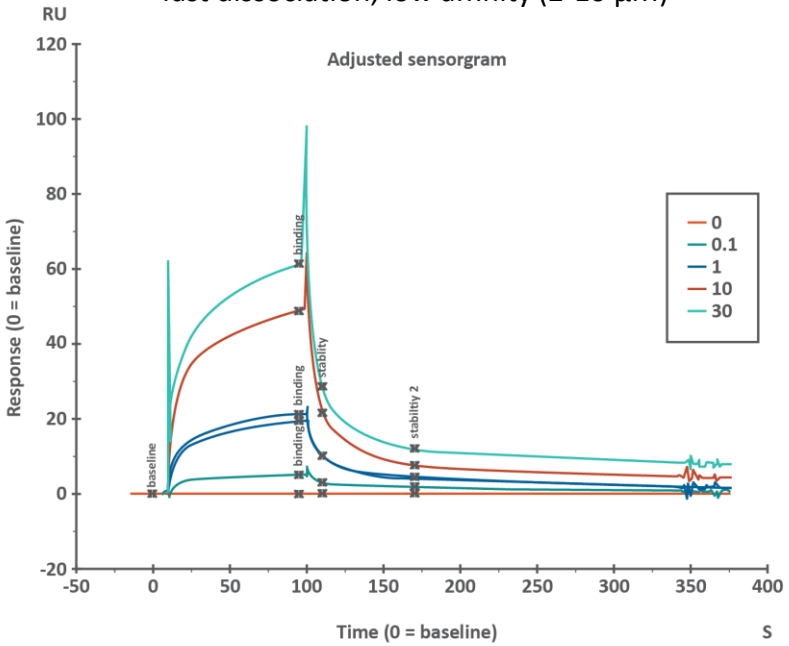
CD16a

fast dissociation, low affinity (ND)



CD64

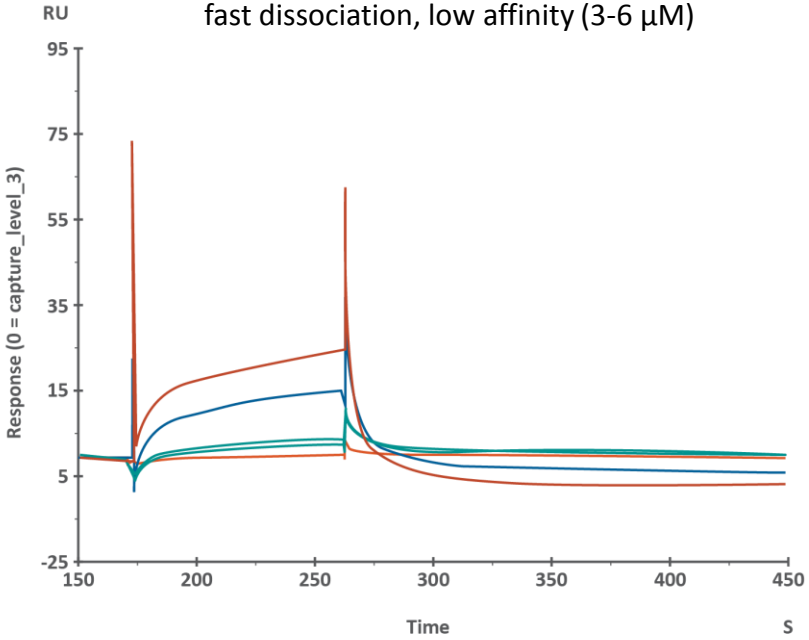
fast dissociation, low affinity (2-10 µM)



Effector Function

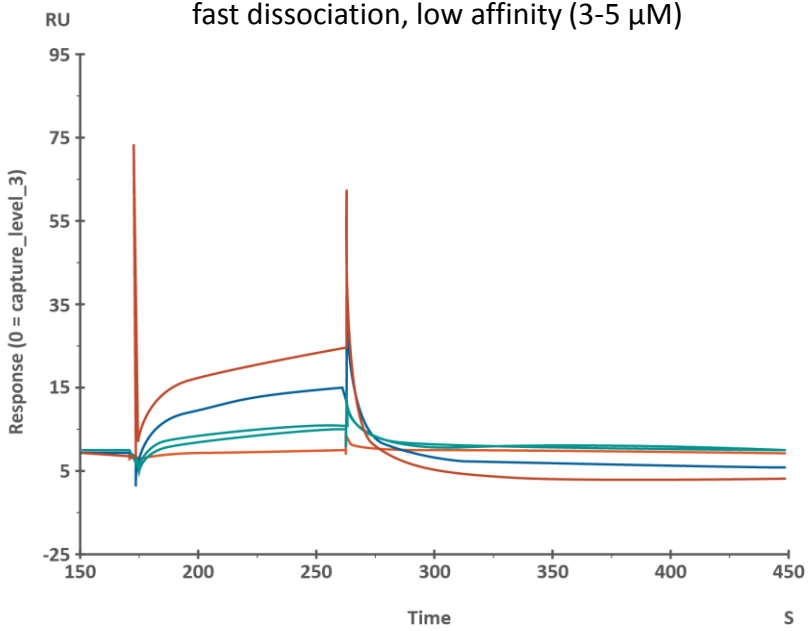
CD32a

fast dissociation, low affinity (3-6 µM)



CD32b/c

fast dissociation, low affinity (3-5 µM)



Biosimilarity Assessment

	HER2	CD64	CD16a		FcRn
Analyte	K_D [pM]	K_D [nM]	K_D1 [nM]	K_D2 [nM]	K_D [nM]
Trastuzumab	195	6	325	17	76
Biosimilar A	135	6	160	36	77
Biosimilar B	140	6	270	36	160
Biosimilar C	165	6	250	37	50

BioAgilytix

Thank You for Your Attention!