



WhitePaper

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Generating a Quality Attribute Profile for Antibody-Based Biosimilars: Assessing Differences in Fc-Associated Effector Functions

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Abstract

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During biosimilar drug characterization, the use of orthogonal methods is necessary in providing a complete, detailed overview of the molecule being assessed – Surface Plasmon Resonance (SPR) assays allow for the description of an interaction by both kinetics and affinity, and are able to generate a wealth of information per sample assessment. At Sartorius Stedim BioOutsource, we are developing and have developed a number of SPR assays which use Biacore instruments to assess binding of drug molecules to target molecules, FcRn, and Fcγ-Receptors, and are able to provide an overview of the performance of a biosimilar molecule.

Here, we review the use of the Sensorgram Comparison tool of the Biacore T200 software in two separate case studies, to detail instances where the affinity (KD) measurements and the binding responses did not sufficiently describe the drug substance interaction to the associated ligand. Sensorgram comparison was then used to give an additional level of comparability to the original data, resulting in a better understanding of the correlation between functional and physiochemical results. This white paper summarises the information from a talk given by Dr Graeme Anderson, at DIPIA in 2018.

Generating a Quality Attribute Profile for Antibody-Based Biosimilars: Assessing Differences in Fc-Associated Effector Functions

The application of orthogonal methodology in biosimilar characterization is required by regulators and provides a complete and detailed overview of the molecule being tested. Surface Plasmon Resonance is an important, critical, and reliable technology, which can be used in conjunction with other methods to describe Critical Quality Attributes (CQAs) which guide process development.

However, not all instances of drug substance characterization are accurately described using the affinity (KD) measurements and the binding responses generated by Biacore T200 and Biacore 4000 instruments. There are a number of examples where an additional level of comparability would be beneficial:

- The association and dissociation rates of an analyte binding to a ligand can be different to that of another analyte, however, these differences can be missed if the affinity measurements (Equilibrium dissociation constant, KD) are determined to be similar to each other. How can we quantify differences in the association and dissociation rates? How can we confirm that the kinetics of the interaction are comparable alongside the generated KD?
- The kinetic and affinity models available on the Biacore software do not always accurately represent the complexity of an interaction – How can we demonstrate similarity of the interaction kinetics without an adequate model to fit the data to?

Our SPR team are able now to describe differences in sensorgram curves from Biacore T200 instruments, which can provide clients with extra confidence in the results, and can also provide a link between results from orthogonal methods. We have carried out two case studies where sensorgram comparison has been a useful and informative tool in the characterization of mAb CQAs, and has demonstrated correlation between functional and physio-chemical results.

Case Study 1: Truxima® versus MabThera® Glycan Profiles and Functionality

Biosimilar Truxima® and innovator MabThera are used for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukaemia, and rheumatoid arthritis. They bind to CD20 on the surface of B cells, mediating ADCC through binding of FcγRIIIa, and enhanced macrophage activity through binding of FcγRIIIa.

Research on the activity of monoclonal antibodies has shown that post-translational addition of sugar molecules can be key modulators in the functionality of a drug substance (1). Therefore, the N-glycosylation profile is a commonly described CQA for therapeutic antibodies.

A combined physiochemical and binding | functional bioassay set up was used to define the CQAs for these molecules. N-glycan profiles, ADCC profiles, and binding to Fc-γ receptor IIIa (CD16a) of biosimilar Truxima® and innovator MabThera® were investigated.

Glycan Profiles

The LC-MS demonstrated a reduced afucosylation level in the biosimilar Truxima® compared with the innovator:

Molecule	Protein Molecular Weight	Glycan Profile A-fucosylation Level
MabThera®	As Expected	6.80%
Truxima®	As Expected	4.40%

Table 1: LC-MS data – the level of a-fucosylation for each molecule analysed.

The literature suggests (2) that this reduced afucosylation level would have an impact on the functionality of the mAb, specifically due to a decrease in the association rate to FcγRIIIa.

ADCC and SPR results

From the initial SPR assessments, the KD of the interactions (the equilibrium dissociation constant between mAb and FcγRIIIa) were calculated using the 1:1 Langmuir Binding Model. Using this KD value, a Relative Affinity value (Relative Affinity (%) = $(KD_{RS}/KD_{Sample}) \times 100$) and a Relative Binding value of the biosimilar Truxima® against a reference standard preparation of MabThera® were calculated.

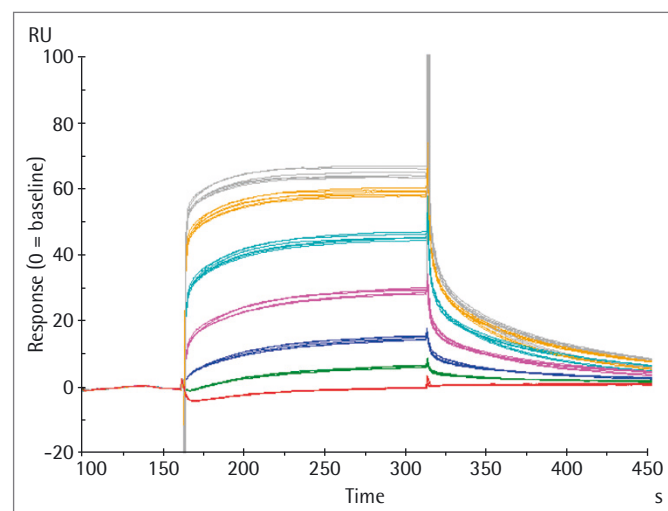


Figure 1: Overlay of sensorgrams for both MabThera® and Truxima® concentration series, taken from Biacore T200 version 3.0 software.

Sample ID	Molecule	SPR – FcγRIIIa	
		Relative Affinity (%RS)	Relative Binding (%RS)
RS	MabThera®	100	100
SA	Truxima®	106.5	108.2
QC	MabThera®	100.4	108.3

Table 2: Overview of the relative affinity and relative binding results generated from the analysis of the sensorgrams in Figure 1. Results for SA and QC given as a percentage against the RS material.

Both Relative Affinity and Relative Binding results for Truxima[®] suggest that there are no differences between the innovator and the biosimilar material, which does not correlate with our assessment of the Glycan profiles or the literature.

The relative potency ADCC result also suggested no difference between the innovator and biosimilar material, with their Relative Potency compared with the reference standard from this assay calculated as 97.0% and 98.0%, respectively.

From this data, is there any way that we can demonstrate differences in the performance of the materials that corresponds with our Glycan profiles and with the literature?

Using Sensorgram Comparison

Our ADCC assay and our Relative Binding measurements give one measurement at one point of time – the end point of the assay. Consequently, they give us only a snapshot of overall molecule performance.

The Affinity measurement is a single value calculated from one association rate and one dissociation rate – this is slightly more indicative of differences across the whole assay, however, this single value can mask differences in the kinetics of an interaction where similar KD values are obtained from different k_a and k_d values.

Sensorgram comparison, a tool available in the Biacore T200 Evaluation Software (version 3.0 and above), compares sensorgram curves at multiple points along the curve. Upon setting a Reference Standard (i.e. in this case, MabThera[®]), the software defines a reference curve, with an upper and lower standard deviation limit – sample curves are then assessed as being within these boundaries at each point, and the sample is given a similarity score (%) compared to the Reference Standard preparation.

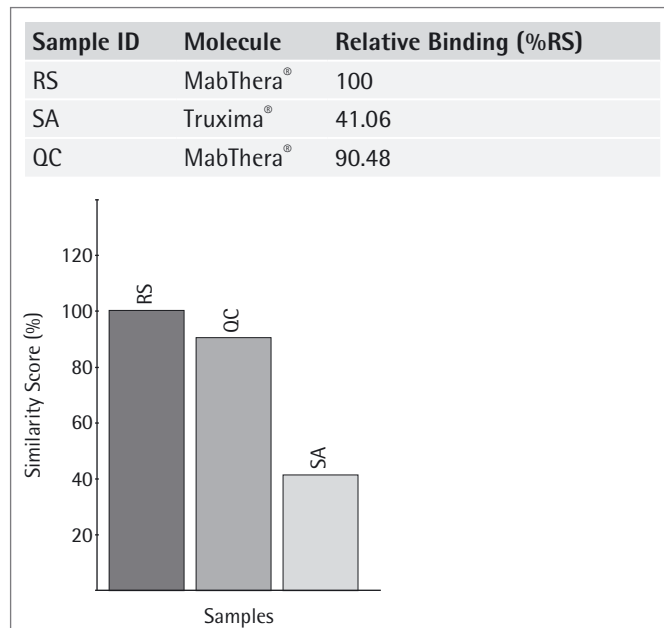


Figure 2: Overview of the sensorgram comparison results. From the bar chart and the table, the Reference standard and QC Reference standard samples are similar, but the Truxima[®] sample returns a similarity score of 41.06% compared with the Reference Standard preparation.

Using Sensorgram Comparison, we can see that although the biosimilar returned a similar Relative Affinity result to the innovator, the overall shape of the sensorgrams is different. This suggests that the k_a and k_d values are measurably different between the innovator MabThera[®] and the biosimilar Truxima[®].

Here, the use of sensorgram comparison has been shown to be a useful additional tool in providing a fuller description of the interaction.

Case Study 2: Humira[®] versus Biosimilar Candidate: C1q Binding by SPR

Humira[®] is a fully human monoclonal IgG1 antibody which is used for the treatment of numerous autoimmune diseases. It binds to human tumour necrosis factor alpha (TNF α) and neutralises its mechanism of action by blocking its interaction with cell surface TNF receptors. It thereby activates the classical complement pathway through an initial interaction with C1q leading to a signalling cascade which culminates in complement-dependent cytotoxicity (CDC) and destruction of target cells.

LC-MS, CDC, and SPR results

Binding of Humira[®] and the biosimilar candidate to C1q was assessed through a newly developed SPR method on the Biacore T200. C1q is a hexameric molecule with six Fc-binding sites, and due to this complex structure, none of the kinetic models available on either the Biacore T200 or Biacore 4000 accurately describe the interaction. Therefore, with the SPR method, we focussed initially on the Relative Binding measurement.

Assay	Humira [®] (RS material)	Biosimilar Candidate (Sample material)
Glycan Profile	G1 16.0%, G2 1.0%	Increased Galactose: G1 43.5%, G2 7.9%
Relative Potency CDC Assay	100.0%	123.9%
Relative Binding (%RS) SPR	100.0%	151.8% (Replicate 1) 156.5% (Replicate 2)

Table 3: Overview of the glycan profile data, CDC data and Relative binding data between the Humira[®] material and the Biosimilar candidate, demonstrating higher galactose, higher relative potency and higher relative binding of the Biosimilar candidate compared with the RS.

From the combined data from the orthogonal methods, the increase in terminal galactose in the biosimilar candidate corresponds with functionality – specifically, increased binding to C1q compared with Humira[®] and therefore increased ability to induce CDC activity.

However, with the lack of kinetic comparability, and with the results given above all being single point of assay results, it would be beneficial here to use sensorgram comparison to confirm that the difference between Humira[®] and the biosimilar candidate can be highlighted at all points through the sensorgram.

Using Sensorgram Comparison

By visual examination of the sensorgrams alone, there is a clear difference between the Humira[®] (green sensorgrams) and the biosimilar candidate at the same concentration (red sensorgrams), which demonstrate the increased relative binding result of the biosimilar candidate.

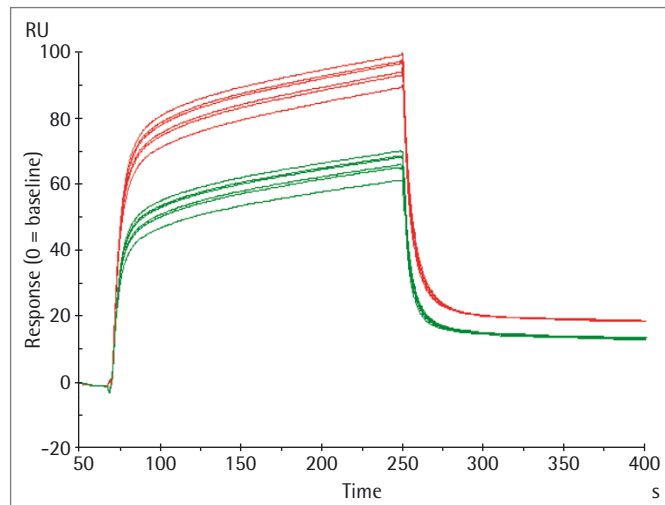


Figure 3: Sensorgrams representing the increased binding response (RU) of the biosimilar candidate (Fc 2-1, red sensorgrams) compared with Humira (Fc 3-1, green sensorgrams).

Using sensorgram comparison to assess multiple points along the sensorgrams, we can assign a value to this visual interpretation of the data, and determine similarity of the sensorgrams in the absence of an appropriate kinetic model. In doing so, it was quantifiably determined that the sensorgrams for each replicate of the Biosimilar candidate (Sample A), were different to the Reference Standard along multiple points on the curve, adding further evidence of a difference between the two molecules in the absence of kinetic data.

Sample ID	Test Material	Similarity Score (% compared to RS)
Reference Standard (RS)	Humira [®]	100.00
Sample A (replicate 1)	Biosimilar Candidate	59.58
Sample A (replicate 2)	Biosimilar candidate	66.11
QC Reference Standard	Humira [®]	94.77

Table 4: Sensorgram comparison results for the biosimilar candidate when compared with Humira[®]. The comparison is quantified by a similarity score, giving the biosimilar candidate results as a percentage of the RS material.

In the absence of an appropriate kinetic model, sensorgram comparison has allowed us to quantify differences in the performance of two different molecules, adding to results gathered from orthogonal methods to give a complete overview of the biosimilar candidate.

Conclusions and Future Uses

As demonstrated above, sensorgram comparison can be a useful and informative tool in the generation of a complete and detailed overview during biosimilar characterization, adding to the wealth of information produced from Surface Plasmon Resonance assays. In particular, sensorgram comparison can help to quantify similarity in the absence of an appropriate kinetic or affinity model, to demonstrate differences between sample replicates, and can help to confirm differences in the kinetics of interactions which have been masked by the generation of one overall affinity measurement (Equilibrium dissociation constant, KD).

This tool can be used to analyse Biacore T200 data either alongside the generation of relative affinity and relative binding measurements, or can be used retrospectively to investigate differences in results from an assay and to help explain data between orthogonal methods.

Our SPR team are able to offer sensorgram comparison as an add-on analysis in Biacore T200 assays, providing clients with extra confidence in their results and a more complete overview of their biosimilar performance.



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