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Development of a Target Binding SPR Assay for Therapeutic Antibodies and Fc Fusion Proteins

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1. Introduction

Sartorius has developed a platform Surface Plasmon Resonance (SPR) method to measure the target binding of monoclonal antibodies (mAbs) to their targets. This method can test up to 12 samples in a single run, which takes around two and a half days – a far higher throughput than previous SPR methods, which immobilized the target. The binding of therapeutic antibodies to their target molecule is critical for the efficacy of mAb drugs. A variety of method can be used to assess the binding of antibodies to their target(s). SPR is a real-time, label-free method that can be used to assess binding kinetics (on and off rates) and binding responses from interactions between drug molecules (e.g., mAbs and their target). Binding kinetics can reveal differences in antibody target binding, which may not be visible by end point assays, as antibodies with markedly different kinetic parameters can show the same affinity for a target.

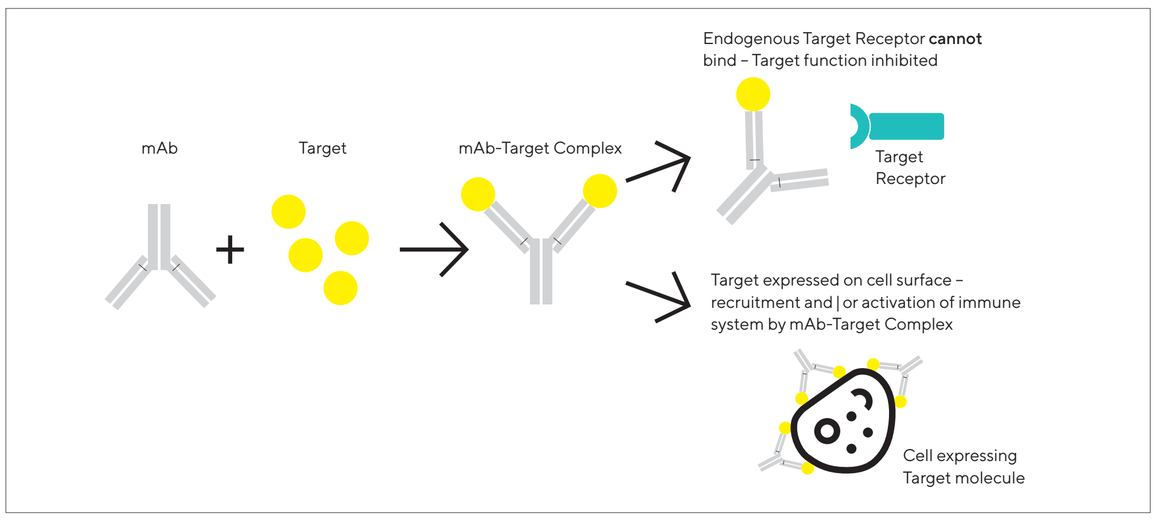

Figure 1: Schematic diagram of antibody target binding and impact on drug function.

2. Experimental Setup

This assay utilizes a capture-based methodology. The SPR sensor chip is coated with a capture molecule specific to the Fc region of mAbs. The reference surface is also coated with a capture molecule to allow reference subtraction. The mAb or Fc-containing drug (such as Fc fusion proteins) is then captured on the active surface, and a stable baseline is established by washing the surface with a running buffer. The mAb target is then injected over the capture mAb for a designated time and then allowed to dissociate in the running buffer. The surface is then regenerated between each target assessment, and a fresh capture of the mAb is performed, followed by the next concentration of the target. Repeated injections allow the generation of a series of sensorgrams for each sample. The data are analyzed using a 1:1 Langmuir kinetic model, and binding data are imported for parallel line analysis of samples against the reference standard.

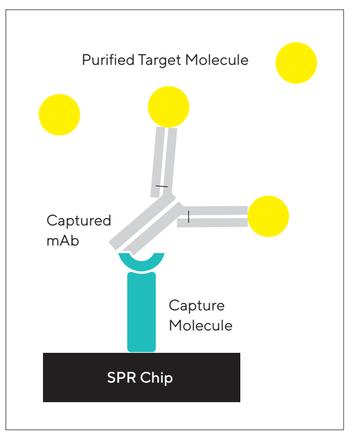

Figure 2: Schematic of the SPR platform assay for assessing mAb target binding.

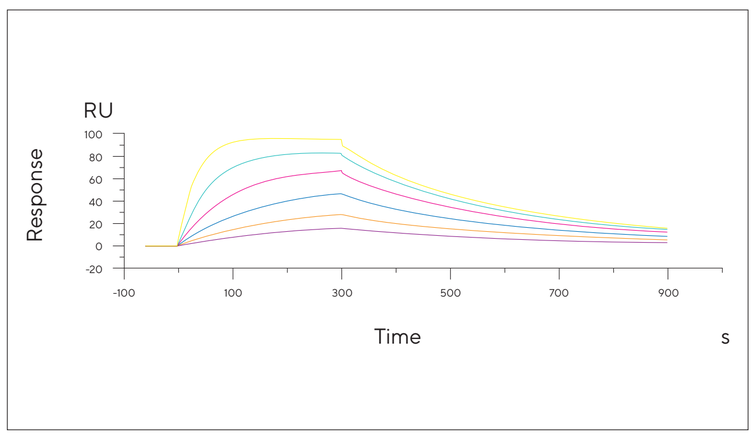

Figure 3: Typical sensorgram series for target binding.

3. Testing the Platform Method for Versatility

A variety of mAbs and a Fc fusion protein were tested using the experimental setup (shown above in Figure 2) to assess the versatility and ease of developing this assay platform. The following mAbs and their corresponding targets were tested using the platform method:

mAb of Interest	mAb Isotype	Molecular Target	Used in Treatment of
Nivolumab	IgG4	PD1	Various Cancers
Pembrolizumab	IgG4	PD1	Various Cancers
Atezolizumab	IgG1	PD-L1	Various Cancers
Durvalumab	IgG1	PD-L1	Various Cancers
Trastuzumab	IgG1	Her2	HER2 + Breast and Gastric Cancers
Omalizumab	IgG1	IgE	Asthma, CIU, and Nasal Polyps
Abatacept	CLTA4-Fc Fusion	CD80 and CD86	Arthritis (various)

Table 1: List of drug-target pairs tested using the capture platform method.

4. Results – Panel of mAbs Binding to Targets

All of the mAbs in Table 1 were assessed using the platform SPR method, and representative sensorgram series are shown below. Kinetic fits were performed on the data using a 1:1 Langmuir fitting model (except for CD86, which used the heterogeneous ligand model). Several things can be noted from the sensorgrams: high- quality fits are seen with good agreement between the model fit versus the raw data, and, the assay is sensitive and detected differences in kinetics between mAbs that target the same molecule and mAbs, which target different molecules.

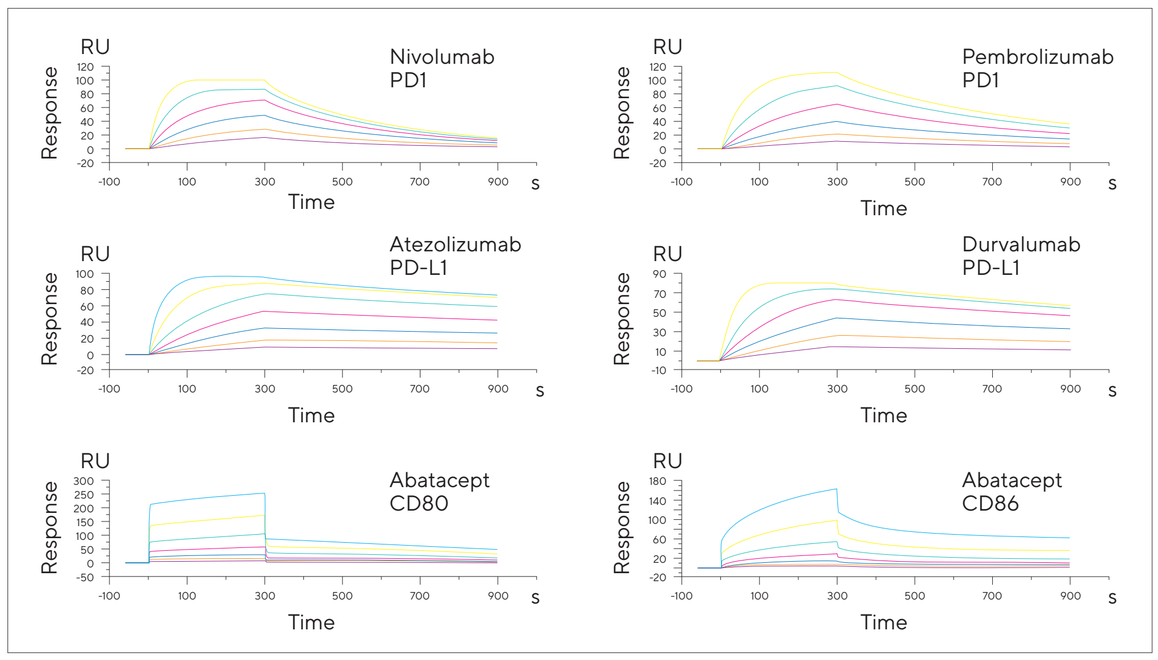

Figure 4: Sensorgrams from a panel of mAbs binding to their target molecules.

5. Results – Pembrolizumab Assay Performance

Three consecutive runs were performed using Pembrolizumab and PD-1 to assess assay performance with respect to relative affinity and relative binding: accuracy, intermediate precision, and specificity.

Parameter	Criteria	Range Qualified for	Notes
Relative Affinity Accuracy	<20% from expected value	70-143 %	Maximum inaccuracy was 4.1%
Relative Binding Accuracy	<20% from expected value	70-143 %	Maximum inaccuracy was 5.9 %
Relative Affinity Intermediate Precision	<20% CV of replicates	70-143 %	Highest %CV was 3.7 %
Relative Binding Intermediate Precision	<20% CV of replicates	70-143 %	Highest %CV was 3.3 %
Specificity	Isotype matched, but does **not** target specific antibody – does **not** bind to target	N | A Passed	Natalizumab did not bind to PD1 when captured on chip

Table 2: List of drug-target pairs tested using the capture platform method.

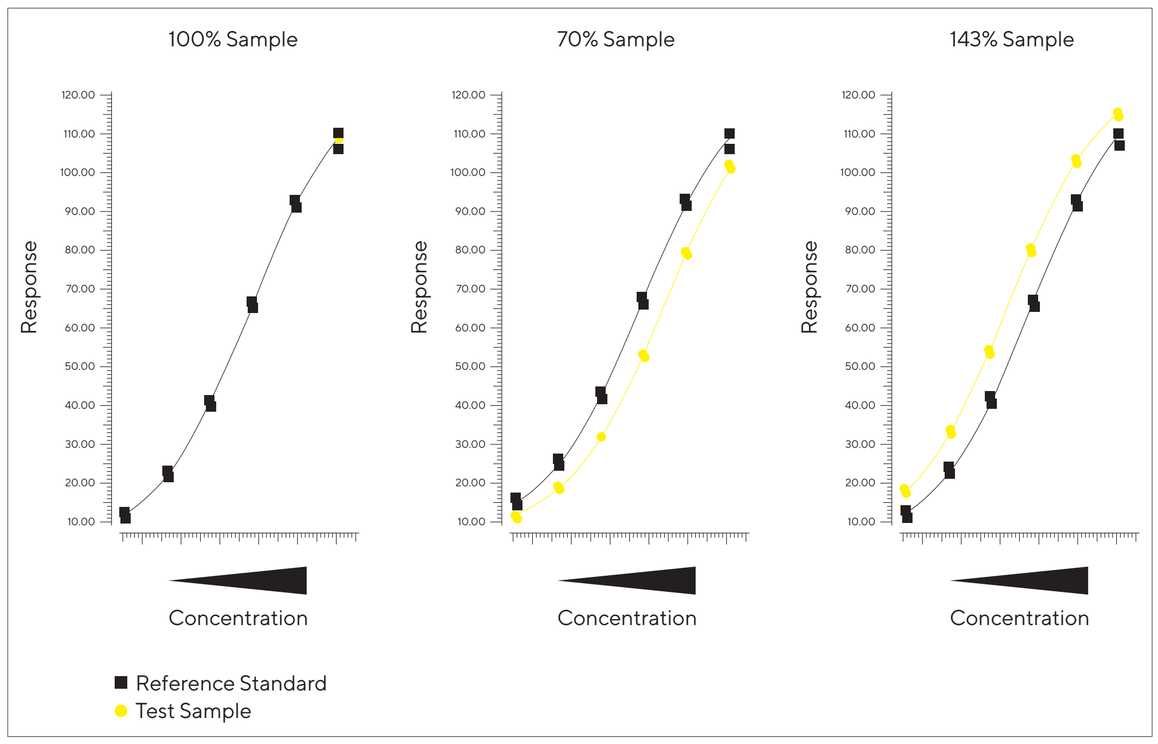

Figure 5: Parallel line analysis of Pembrolizumab accuracy samples for binding to PD-1.

6. Conclusion

The data presented here demonstrates a versatile, accurate, and specific SPR capture methodology for assessing the kinetics and binding of various mAbs to their target molecule. Fc-containing molecules such as Fc fusion proteins can also be assessed using this method as well – demonstrated by testing Abatacept. The relatively high throughput of 12 samples per run reduces the turn around time for sample testing and decreases operational costs. This method can be transferred between different SPR systems with minimal development to further increase throughput or fit to local instrumental and operational constraints.