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Profiling antibody polyreactivity and polyspecificity using HT-SPR on the Carterra® LSA®

Key Takeaways

- Monoclonal antibodies are an important class of therapeutic proteins used to treat a wide range of diseases
- Having a better understanding of the polyreactivity and polyspecificity of potential monoclonal antibody candidates can result in more effective and efficient development of therapeutics
- The LSA, which runs HT-SPR, can be used to fully automate large scale, real-time screening of panels of antibodies to gain deep information on their specificity or off-target binding during early development

Introduction

Monoclonal antibodies (mAbs) are one of the most important classes of therapeutic proteins, which are used to treat a wide range of diseases (e.g., oncology, inflammation, and autoimmune diseases). Their use as therapeutics is supported by their ability to bind specifically to their target, have long serum half-lives, and have high tolerability due to the abundance of IgG in the body. Despite these beneficial characteristics, antibodies still regularly fail during therapeutic development. One of the factors causing these failures is linked to polyreactivity and polyspecificity[1-4]. Both terms refer to the ability of an antibody to bind to several targets and in the context of drug development to off-target binding. While polyreactivity can be defined as chemical "stickiness," polyspecificity describes the ability of the antibodies to bind specifically to structurally related epitope on different antigens. Either of these off-target activities can potentially lead to poor pharmacokinetics (PK), potency, bioavailability and/or immunogenicity[5]. Being able to investigate antibody specificity in early discovery is necessary to mitigate costly failure during development phases[6].

Here, we describe a high-throughput surface plasmon resonance (HT-SPR) assay to profile antibody polyreactivity/polyspecificity. This assay takes advantage of the LSA's ability to monitor binding interactions of an analyte against 384 ligands in an array. A selection of proteins reflecting the human blood biological environment, proteins displaying specific, biophysical properties (highly glycosylated and low PI) and positive controls were included. In addition recombinant antibody target protein, in this case PD-1, was used to understand polyreactivity/polyspecificity. Subsequently, a fully automated assay enabling the characterization of a panel of antibodies in a single run was carried out. The assay automates several steps including the capture of the protein selections, the injection of the antibodies at two concentrations and the regeneration of the biosensor. The binding levels to the various target proteins were compared to assess the levels of an off-target binding.

Materials and Methods

Material

The following his-tagged proteins were purchased from Abcam and Sinobiological: Serum albumin (Abcam Cat.# ab217817), Fibrinogen (Abcam Cat.# ab202200), α-1-antitrypsin (Abcam Cat.# ab276242), Mannan binding lectin (Abcam # ab229367), α-1-acid glycoprotein (Sinobiological Cat.# 16030-H08H), α-1-fetoprotein (Sinobiological Cat.# 12177-H08H), α-2-macroglobulin (Sinobiological Cat.# 10952-H08B), β-2-microglobulin (Sinobiological Cat.# 11976-H08H), Haptoglobin (Sinobiological Cat.# 80534-R08H), Complement component 3 (Sinobiological Cat.# 13182-H08H), Transferrin (Sinobiological Cat.# 11019-H08H), Protein A (Abcam Cat.# ab52953), Pepsinogen (Sinobiological Cat.# 12072-H08H), Erythropoietin (Sinobiological Cat.# 90133-C08H).

Polyreactivity/Polyspecificity Monitoring Assay

For the assay, an HC200M biosensor (Carterra Part # 4287) immobilized with an anti-His antibody (Genscript Cat.# A00186) was used. Within the Navigator control software, the Capture Kinetics application was applied to characterize the binding of the antibodies to the immobilized proteins. His-tagged proteins were prepared at 50 nM in HBSTE buffer (Carterra Part # 3630) and captured in duplicate onto the biosensor for 20 min. Protein A and antigen of interest were used as positive controls. Antibodies were diluted to 10 nM and 100 nM in HBSTE buffer and injected over the protein array for 5 min. Finally, surface of the biosensor was regenerated using 3 x 90 s pulses of 10 mM glycine pH 1.5 (Carterra Part # 3639).

Data Evaluation

Sensorgrams were double referenced, y-aligned, and binding level report points were taken at the end of the association phase using the Carterra's Kinetics software, **Figure 1** and **Figure 2**. Additional data processing was performed with Microsoft Excel. For each antibody, the ratio of signal over theoretical Rmax, calculated from the level of immobilization of a protein, was determined to assess the binding specificity. These binding ratios were reported in **Figure 3** and evaluated using a gradient color scheme. Specific antigen binding and Protein A binding were shown in graded purple scale from white to dark purple, where dark purple represents >80% of the Rmax. For all other proteins, binding signals up to 15% of the Rmax were considered as noncritical and were shown in green. Signals from 15% to 25% represent a low to moderate unspecific binding and were shown with a graded color scale from green to yellow. Higher signals, >25%, were shown in red and represent significant nonspecific binding to the respective protein.

Results

In the present work, we describe the development of an HT-SPR assay for early assessment of antibody specificity during discovery. This assay uses a biosensor derivatized with an anti-His antibody capable of capturing a predictive protein panel. The rationale for the protein selection was their abundance in blood, increasing the probability of off-target binding if the investigated antibody displays a certain degree of affinity for a specific blood protein. It is worth noting the composition of the protein selection here can be expanded or modified to reflect additional biological compartments. Protein A and specific antigen as positive controls were included in the panel.

All antibodies bound well to their respective targets and Protein A. Two antibodies, (Ab 7 and Ab 9) showed measurable levels of off-target binding to several of the non-target proteins and could have high risk or be questionable for further development. The other antibodies had clean off-target profiles and appear to have no demonstrated polyspecificity.

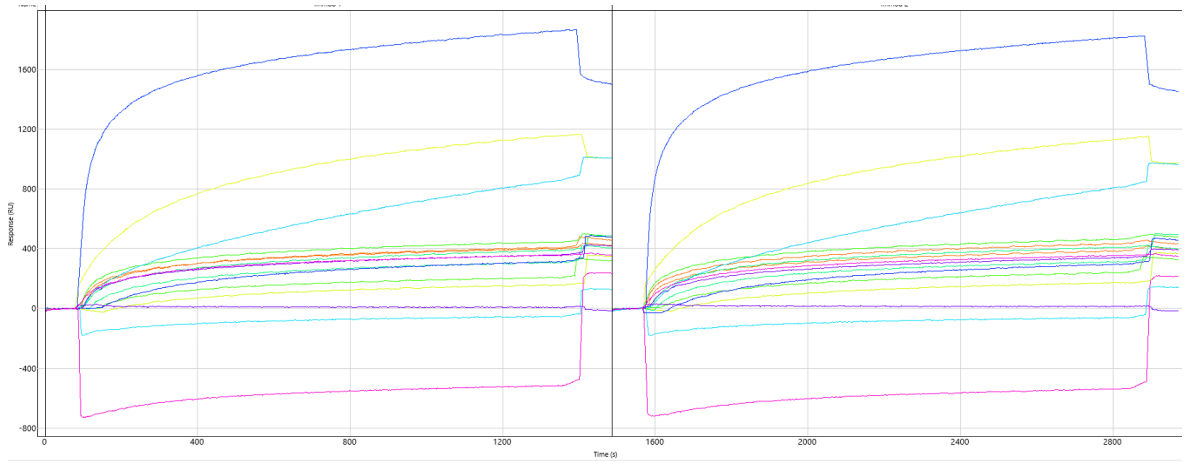

Figure 1. Arraying of proteins in duplicate at 50 nM on the HC200M biosensor surface. The levels of immobilization were determined to calculate the theoretical maximum binding capacity of each spot. Immobilization levels were then used in conjunction with Rmax to assess the polyreactivity of each antibody to the respective protein.

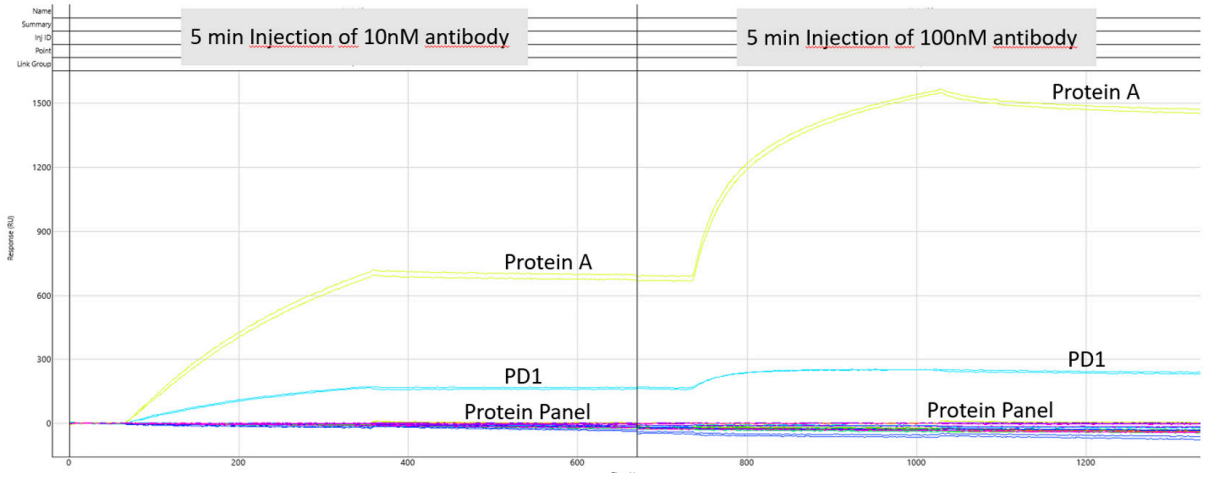

Figure 2. Sequential injections of an anti-PD1 antibody at 10 nM and 100 nM across the protein array.

	Anti-RBD mAbs									
	Ab1-10	Ab1-100	Ab2-10	Ab2-100	Ab3-10	Ab3-100	Ab4-10	Ab4-100	Ab5-10	Ab5-100
RBD	0.22	0.41	0.16	0.35	0.35	0.41	0.24	0.35	0.22	0.36
PD1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Protein A	1.56	2.70	1.68	3.74	1.10	2.36	1.01	2.27	1.90	3.20
α-2-macroglobulin	0.02	0.03	0.03	0.04	0.02	0.05	0.03	0.00	0.07	0.05
α-1-acid glycoprotein	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Pepsinogen	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.01
Serum albumin	0.01	0.02	0.00	0.00	0.00	0.01	0.01	0.02	0.00	0.00
Haptoglobin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
α-1-fetoprotein	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.01
Erythropoietin	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00
ß-2 microglobulin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Complement component 3	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.02	0.01	0.01
Fibrinogen 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
Mannan binding lectin	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.01	0.00
Transferrin	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01
α-1-antitrypsin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blank	0.18	0.28	0.10	0.08	0.45	0.09	0.20	0.10	0.04	0.02
	Anti-PD1 mAbs									
	Ab6-10	Ab6-100	Ab7-10	Ab7-100	Ab8-10	Ab8-100	Ab9-10	Ab9-100	Ab10-10	Ab10-100
RBD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PD1	0.35	0.36	0.19	0.33	0.36	0.36	0.36	0.36	0.14	0.35
Protein A	1.13	2.58	0.83	2.03	0.87	2.04	0.57	1.69	0.67	1.85
α-2-macroglobulin	0.02	0.04	0.02	0.04	0.02	0.03	0.03	0.03	0.02	0.03
α-1-acid glycoprotein	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Pepsinogen	0.01	0.01	0.21	0.33	0.01	0.01	0.18	0.29	0.01	0.01
Serum albumin	0.00	0.00	0.07	0.07	0.01	0.00	0.01	0.01	0.01	0.01
Haptoglobin	0.00	0.00	0.15	0.23	0.00	0.00	0.12	0.23	0.00	0.00
α-1-fetoprotein	0.00	0.00	0.10	0.15	0.00	0.00	0.12	0.18	0.00	0.01
Erythropoietin	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01
ß-2 microglobulin	0.00	0.00	0.10	0.12	0.00	0.00	0.11	0.16	0.00	0.00
Complement component 3	0.01	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.02
Fibrinogen 1	0.00	0.00	0.22	0.34	0.00	0.00	0.22	0.32	0.00	0.00
Mannan binding lectin	0.00	0.00	0.11	0.14	0.00	0.00	0.08	0.14	0.00	0.00
Transferrin	0.01	0.01	0.01	0.02	0.01	0.00	0.01	0.01	0.01	0.01
α-1-antitrypsin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blank	0.01	0.01	0.16	0.08	0.12	0.20	31.00	58.00	0.06	0.22

Figure 3. Analysis of 5 anti-RBD antibodies and 5 anti-PD1 antibodies. Raw signals for binding of the antibodies to blank spots are shown in "blank" row, row 17. Red color indicates a binding to biosensor matrix. Anti-PD1 antibodies and anti-RBD antibodies were tested at 10 nM and 100 nM in duplicates. Values in rows 1–16 indicate antibody binding signal over theoretical Rmax determined from the level of immobilization of a respective protein ratio. As expected, anti-PD1 antibodies and anti-RBD antibodies bound to their respective targets PD1 and RBD as well as to Protein A, rows 1, 2 and 3. Purple shading indicates binding to target proteins and protein A. Color scheme in rows 4–16 visualize degree of binding of the antibodies to the predictive panel of proteins: green/yellow represents a binding ratio of up to 0.15; yellow/orange is up to 0.25; red is above 0.25.

The SPR assay described here provides critical insights in characterizing the specificity of antibodies during early discovery. The selection of proteins can be expanded up to 384 proteins to reflect a specific biological environment without increasing the assay runtime. It is achievable in a fully automated manner. In contrast to plate-based assays, this assay requires little analysis time.

Summary

It has previously been demonstrated that the LSA allows for the kinetic and epitope characterization of biomolecular interactions in a 384-array format that is well suited for the throughput needs of early discovery workflows. This work demonstrates the use of Carterra's technology to perform a rapid off-target binding characterization providing invaluable information for the investigated antibodies. The LSA's array format enables the immobilization of a large panel of proteins (up to 384) reflecting a specific biological environment. Navigator control software allows the characterization of a panel of antibodies in a fully automated manner. While proteins representative of those found in a blood environment were examined here, this assay can easily be modified to include biomolecules from any number of biological environments and across different species.

What distinguishes this approach from many other formats for screening off-target binding is the ability to monitor real-time binding signals which ultimately can be used to quantitate strength of binding. This may allow for more nuanced understanding of the degree of off-target binding and whether it is likely to have biological implications, particularly when only a weak interaction is observed. Additionally, the superiority of the LSA for performing epitope binning allows for off-target binding of candidates to be evaluated in the context of epitope, providing valuable insight into off-target mechanisms.

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