Quick Antibody Off-target Binding Assessment Via High-Throughput SPR

Introduction

Antibody off-target assessment, or polyreactivity assessment, is a crucial test to ensure the specificity and safety of therapeutic antibodies. Polyreactivity testing involves evaluating whether an antibody binds specifically to unintended targets. Off-target binding and poor specificity could lead to adverse effects including triggering unwanted immune responses, which can lead to tissue damage or other toxic effects. Off-target binding can also lead to poor pharmacokinetics, where the antibody is sequestered broadly, reducing its availability to bind the intended target [1,2,3,4]. Addressing off-target binding is crucial for the successful application of antibody therapeutics, and by using high-throughput methods, these tests can be incorporated early in the candidate selection process [5, 6, 7].

Here, we describe a high-throughput surface plasmon resonance assay to assess antibody off-target binding, which can be integrated as part of the drug discovery process. The Carterra LSA monitors the SPR signals of up to 384 ligands in a single array, thus allowing the off-target binding profile of a panel of antibodies to be determined against panels of reagents. In this study we chose two common polyreactivity agents, DNA and lipopolysaccharide (LPS). Their particular biophysical and biochemical properties, such as enrichment in negative charge and amphipathic properties, make them both relevant to polyreactivity testing. Additional reagents can be easily added to expand the breadth of the polyreactivity profile.

Methods and Materials

Selection of Antibodies and Probes

Antibodies targeting PD1 produced in IgG1 format by Adimab [8], and polyreactive antibodies Bococizumab and Briakinumab, were provided by Ichnos Sciences. The evaluated probes were: DNA, MB-grade from fish sperm (Sigma # 11467140001) and Lipopolysaccharides from *Escherichia coli* O111:B4 (Sigma # L5293).

Polyreactivity Monitoring Assay

For the assay, an SAHC30M biosensor (medium capacity streptavidin biosensor) immobilized with an anti-Fc VHH (CaptureSelect™ Biotin Anti-IgG-Fc (Multi-species) Conjugate # 7102852100) was used. The sensor chip was placed in an LSA^{XT} instrument, primed with HBSTE running buffer, and conditioned with one-minute injections of 20 mM NaOH + 1 M NaCl and 10 mM Glycine pH 2.0. The anti-Fc VHH was diluted into HBSTE buffer to a concentration of 20 µg/mL and injected for 15 min over the biosensor surface. This resulted in immobilization of an average of 1300 RU on the ROIs.

The capture kinetics wizard was used to characterize the binding of the polyreactivity reagents to the captured antibodies. The ligands were diluted in replicates at eight concentrations in a two-fold dilution series ranging from 10 µg/ml to 78 ng/mL. Samples were flowed over the array for 15 minutes and captured via their Fc moieties.

Polyreactivity reagents were diluted at 100 µg/ml and 250 µg/ml in assay buffer and injected over the antibody array for 5 min at 25 °C. Recombinant Human PD-1 (R&D Systems #8986-PD) was used as positive control and injected at 500 nM and 1 µM for 5 min. Finally, the surface of the biosensor was regenerated using two 45-second pulses of 10 mM glycine pH 1.5.

Results

This study provides an example of an SPR-based high-throughput polyreactivity screen using the Carterra LSA^{XT} platform. This assay uses a streptavidin biosensor derivatized with an anti-Fc VHH capable of capturing a panel of human antibodies. The two selected polyreactivity reagents, DNA and lipopolysaccharide (LPS), possess biophysical properties typical of substances targeted by polyreactive antibodies. None of the polyreactivity reagents showed nonspecific binding to the linear polycarboxylate matrix/streptavidin/anti-Fc VHH surface in the absence of captured mAbs.

Figure 1 shows the binding responses for anti-PD1 antibodies. As expected, the antibodies display binding to the PD1 antigen that scales based on the level of antibody captured (ligand concentration). No measurable off-target binding to DNA and LPS was detected, indicating a specific-binding and lower-developability risk.

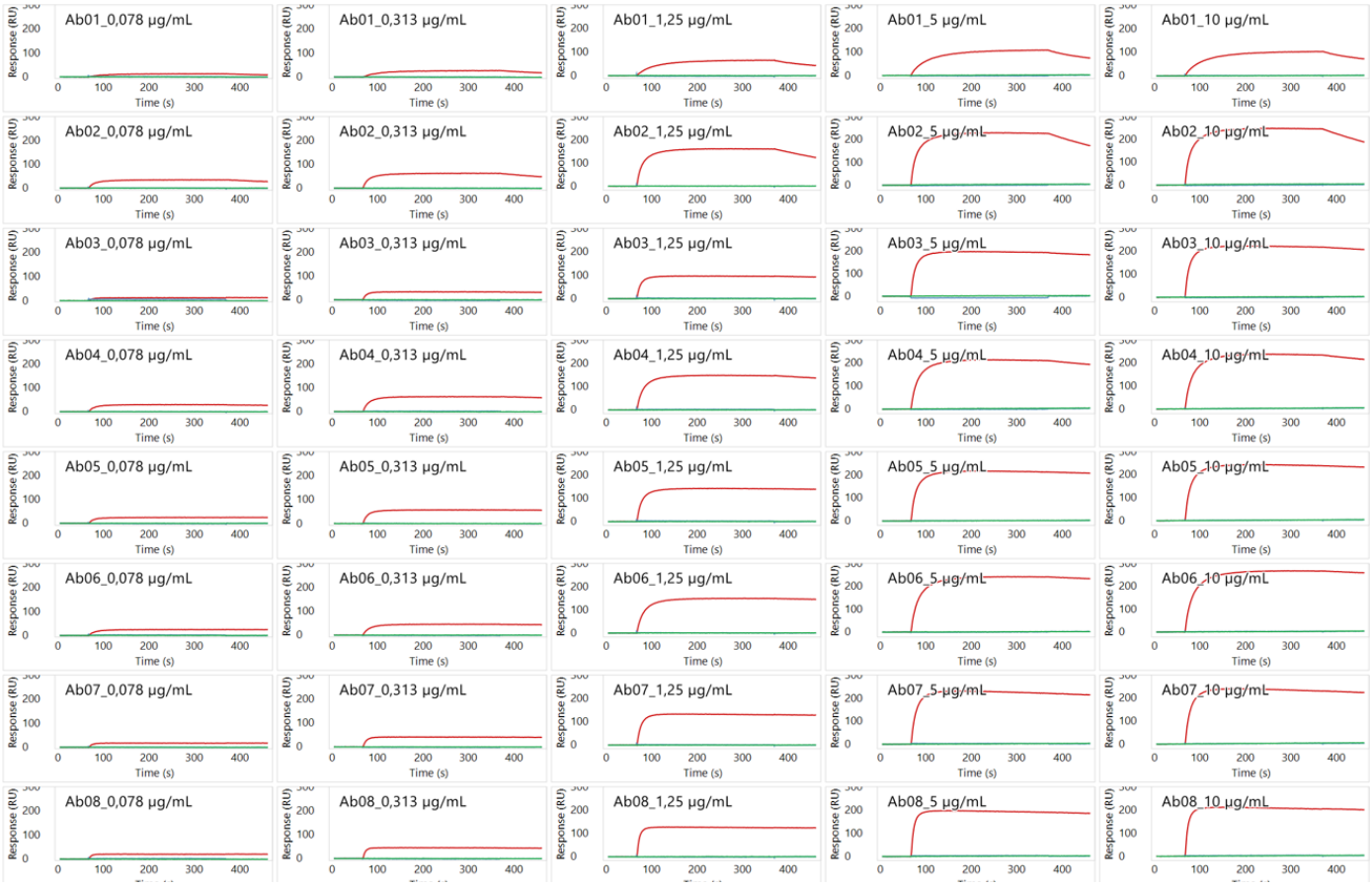


Figure 1. Binding responses of PD1 in red, DNA in green and LPS in blue to captured anti-PD1 antibodies. Only one concentration of each analyte is displayed, 500 nM for PD1 and 250 µg/mL for DNA and LPS.

Bococizumab and Briakinumab demonstrate clear reactivity to both the DNA and LPS analytes with no binding seen to PD1 (**Figure 3**). Both antibodies were selected as positive controls since they were previously reported to be polyreactive in several assays [6, 7]. The scale of polyreactive binding responses are ligand-density dependent confirming that the binding is to the captured antibodies (**Figure 2**).

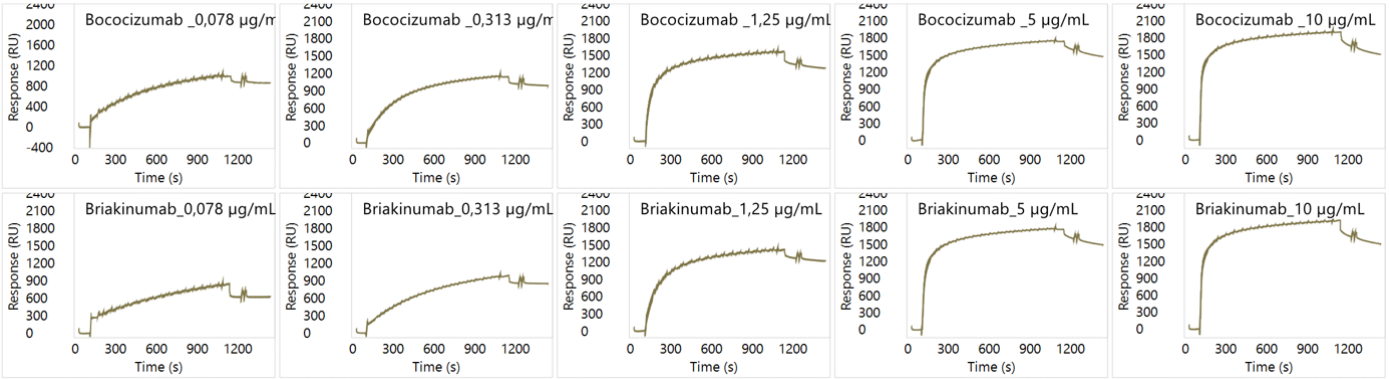


Figure 2. Capture levels of polyreactive antibodies Bococizumab and Briakinumab. Five concentrations of each ligand are displayed.

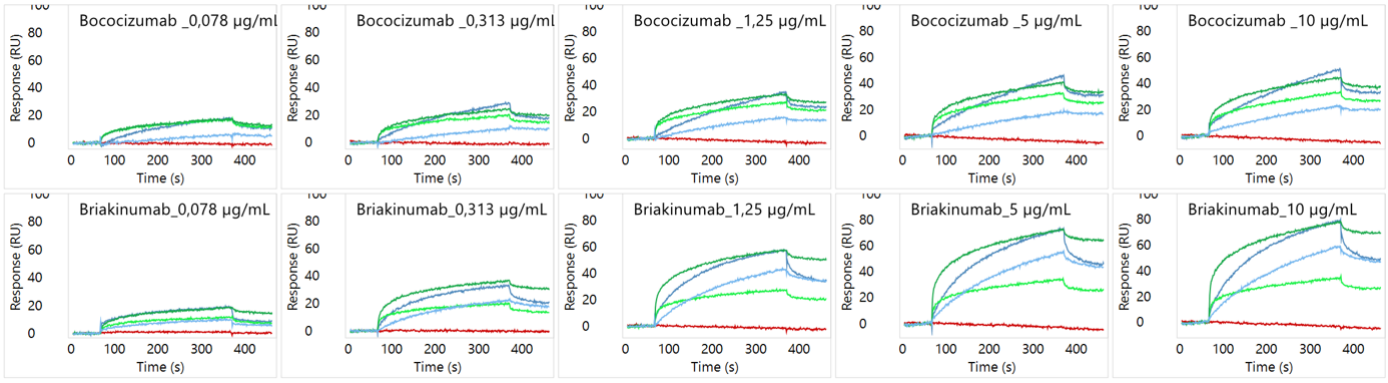


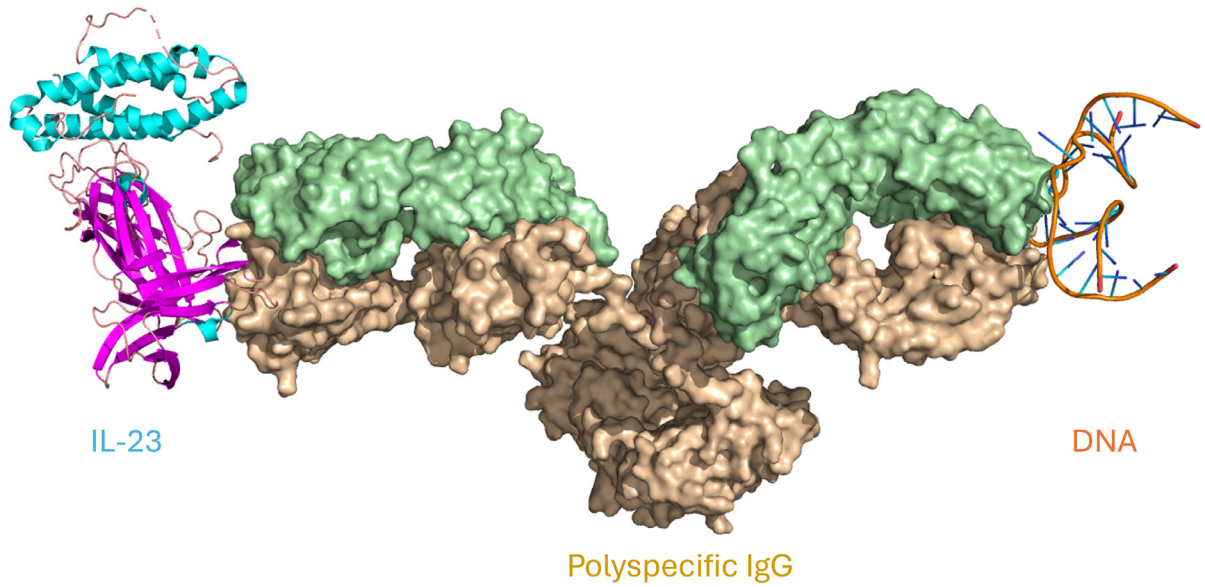
Figure 3. Binding responses of PD1 in red, DNA in green (light green 100 µg/mL, dark green 250 µg/mL) and LPS in blue (light blue 100 µg/mL, dark blue 250 µg/mL) to captured polyreactive antibodies Bococizumab and Briakinumab.

In this study, we demonstrated the binding of two relevant polyreactivity reagents, DNA and LPS, for monitoring off-target polyreactivity for panels of mAbs. We did not observe any nonspecific binding of the two reagents to the biosensor matrix or capture surface, but clear binding was observed to the two polyreactive antibodies used as positive controls. Additionally, we observed that the nonspecific interactions are concentration and ligand-density dependent. Further evaluation is required to determine what polyreactivity reagent signal cut-off should be applied in the process of antibody selection.

Summary

We tested a reduced set of polyreactivity reagents for early off-target binding assessment. Off-target interactions are a critical consideration in drug development since they can adversely affect the performance, specificity, *in vivo* distribution, and half-life of drug molecules. The selection of the polyreactivity markers in this example could easily be expanded. In the described form, this assay can be an integrated part of the drug discovery process and common kinetic-screening workflows. Both DNA and lipopolysaccharide display significant binding signals to polyreactive antibodies while no binding to well-behaved samples was observed.

LSA's array format allows fully-automated polyreactivity assessment on up to 1,152 antibodies, in groups of 384, per run.



A polyreactive antibody is schematized for Briakinumab (V_H wheat; V_L green) binding to the intended IL-23 complex (left; magenta and cyan) and a DNA polyreactivity probe (right; orange ribbon). The figure was generated by overlaying the Briakinumab-Fab/IL-23 complex (PDB: 5NJD) and the Fab/DNA complex (PDB: 5NJD) with the Fab arms of the full length IgG antibody (PDB: 1IGT).

References

1. Van Regenmortel MH. Specificity, polyspecificity, and heterospecificity of antibody-antigen recognition. J Mol Recognit. 2014;27 (11):627–39. doi: 10.1002/jmr.2394.
2. Guthmiller JJ, Lan LY, Fernandez-Quintero ML, Han J, Utset HA, Bitar DJ, Hamel NJ, Stovicek O, Li L, Tepora M, et al. Polyreactive broadly neutralizing b cells are selected to provide defense against pandemic threat influenza viruses. Immunity. 2020;53(6):1230–44 e5. doi: 10.1016/j.immuni.2020.10.005.
3. Stern RA, Trojanowski JQ, Lee VM. Antibodies to the beta-amyloid peptide cross-react with conformational epitopes in human fibrinogen subunits from peripheral blood. FEBS Lett. 1990;264 (1):43–47. doi: 10.1016/0014-5793(90)80760-G
4. Bumbaca D, Wong A, Drake E, Reyes IAE, Lin BC, Stephan J-P, et al. Highly specific off-target binding identified and eliminated during the humanization of an antibody against FGF receptor 4. mAbs. 2014;3(4):376–86. doi: 10.4161/mabs.3.4.15786
5. Cunningham, O., et al., Polyreactivity and polyspecificity in therapeutic antibody development: risk factors for failure in preclinical and clinical development campaigns. MAbs, 2021. 13(1): p. 1999195. doi: 10.1080/19420862.2021.1999195
6. Jain T, Sun T, Durand S, Hall A, Houston NR, Nett JH, Sharkey B, Bobrowicz B, Caffry I, Yu Y, et al. Biophysical properties of the clinical-stage antibody landscape. Proceedings of the National Academy of Sciences. 2017;114:944–49. doi: 10.1073/pnas.1616408114
7. Kelly RL, Yu Y, Sun T, Caffry I, Lynaugh H, Brown M, Jain T, Xu Y, Wittrup KD. Target-independent variable region mediated effects on antibody clearance can be FcRn independent. mAbs. 2016;8(7):1269–75. doi: 10.1080/19420862.2016.1208330.
8. Brown ME, Bedinger D, Lilov A, Rathanaswami P, Vásquez M, Durand S, Wallace-Moyer I, Zhong L, Nett JH, Burnina I, et al. Assessing the binding properties of the anti-PD-1 antibody landscape using label-free biosensors. PLoS ONE. 2020;15(3): e0229206. doi:10.1371/journal.pone.0229206

AN122-1226-2024-REV004

carterra®

questions@carterra-bio.com

Headquarters:
825 N 300 W Suite C309
Salt Lake City, UT 84103

www.carterra-bio.com