Rapid quality control of antibodies using Affinité's simple P4SPR™

Introduction

The quality control of biopharmaceuticals such as antibodies must be performed to ensure quality and safety. For instance, the quality of antibodies is affected by process parameters such as pH, temperature, CO_2, and cell culture metabolites [1]. Not only should these biopharmaceuticals be characterized by standard physicochemical methods such as mass spectrometry, but their biological activities must also be investigated as well. This has traditionally been done using endpoint assays such as Enzyme-Linked Immunosorbent Assays (ELISA) [1] [2]. However, these assays do not provide kinetic and affinity data [1]. An alternative method is Surface Plasmon Resonance (SPR). SPR is a powerful technique to characterize proteins because it is a label-free technique that enables real-time assessment of protein interactions, and it does not require much sample preparation [2]. Large SPR systems have been used to perform protein quality control [2] [3]. Still, access to large centralized SPR equipment is limited, samples must be pre-stored and transported to the facility, and consumable costs are high [4]. Portable SPR devices, on the other hand, can give scientists quick and easy access to obtain valuable kinetic and affinity data needed to validate quality, without compromising sensitivity and specificity.

There have been publications on the use of standard, benchtop SPR to assess the quality of antibodies and other biopharmaceutical proteins [1] [3] [5]. In this application note, we demonstrate the use of our portable SPR device (P4SPR™) to easily determine which source of anti-nucleocapsid antibody exhibits the best binding performance with the SARS-CoV-2 nucleocapsid recombinant protein.

Experimental procedures

A more detailed procedure of the experiment was described in the ChemRXiv paper by Djaileb *et al* [6]. Figure 1 illustrates the detection scheme of anti-nucleocapsid antibody by immobilized SARS-CoV-2 nucleocapsid protein. In general, the gold sensor surface was modified with Affcoat [4]. It was treated with EDC/NHS and then washed with sodium acetate. A SARS-CoV-2 nucleocapsid recombinant (rN) protein solution (10 µg/mL) was added to the surface and was treated for 20 min. It was then again washed with sodium acetate. The protein-modified surface was blocked by 1 M ethanolamine for 10 min (pH 8.5) to reduce non-specific adsorption. It was then equilibrated by the running buffer (RB). Subsequently, an anti-nucleocapsid antibody solution (10 µg/mL) from different sources of antibody manufacturers (Table 1) was manually injected into the sensor, and the SPR responses were collected at each channel (Figure 2, Channels A-C) to yield a triplicate measurement in real-time. A wash of RB and glycine solutions was done before introduction of a new antibody solution. Anti-RBD (receptor-binding domain) was used as control (Figure 2, Channel D) to correct for any fluctuations in temperature and bulk refractive index. The difference in SPR shift was determined from the resonance unit (RU) difference from the beginning to the end of the measurement [6].

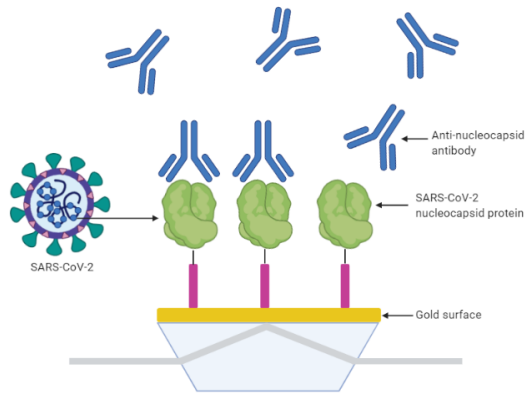

Figure 1 – Detection scheme of anti-nucleocapsid antibody by immobilized SARS-CoV-2 nucleocapsid protein on P4SPR™[1].

[1] Created with Biorender.com

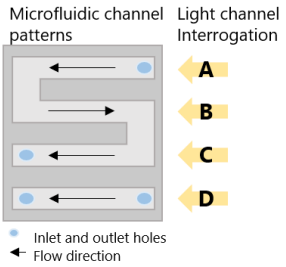

Figure 2 - Schematic of 4-channel microfluidic cell and interrogation channels.

Table 1. Sequence of injection and source of anti-nucleocapsid antibody.

Injection #	Antibody Source
1	AB1, lot A
2	AB1, lot B
3	AB2, lot A
4	AB1, lot C

Results and Discussion

The total time it took for the entire experiment to test 4 different sources of antibody anti-nucleocapsid was just over 2 hours. The average time for each injection was around 18 minutes and the average total time for injection and regeneration was 25 minutes.

Figure 3 shows the SPR response for the first and second source of anti-nucleocapsid antibody in all 3 sample channels. The association phase of anti-nucleocapsid antibody towards SARS-CoV-2 nucleocapsid recombinant protein can be clearly seen.

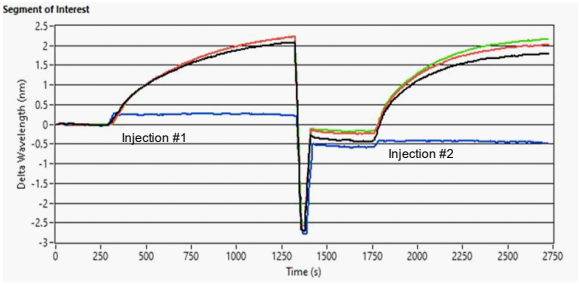

Figure 3. The first and second injections (see Table 1) with simultaneous collection of sensorgrams from each channel (green, red, black). Reference channel is in blue.

Figure 4 shows the average sensorgrams for all four injections.

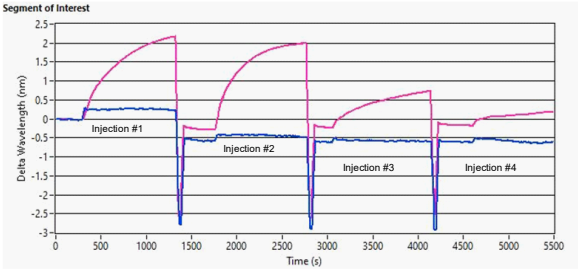

Figure 4. Average sensorgrams of all 3 channels (magenta) from 1st to 4th injection. Reference channel is in blue. Each sensorgram represents a difference source of anti-nucleocapsid antibody (see Table 1).

Figure 5 shows the average responses of the anti-nucleocapsid antibody (i.e. RU intensity in light blue colour) from various sources (in order of injections) in resonance units. It is very clear that AB1, lot B (Injection #2) exhibited the highest SPR response or activity. In addition, it is noteworthy to highlight that the dissociation equilibrium constant (K_d) can still be easily determined from manual injections using increasing concentrations of an antibody or target, for instance [see Application Note #1 – Gene Regulation, Lac Operon/Lac Repressor (DNA-Protein Binding)].

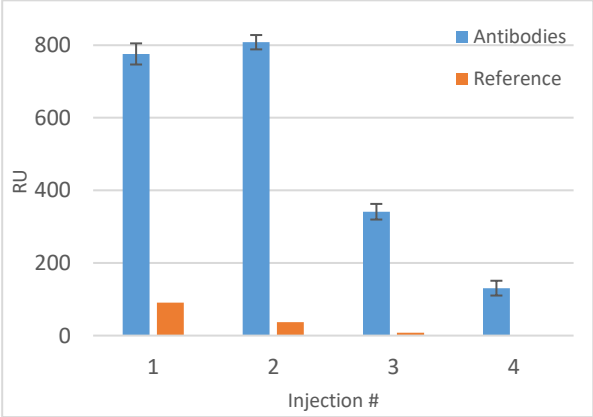

Figure 5. Average shifts in resonance units (RU) for all four sources of anti-nucleocapsid antibody. The reference for Injection #4 is too small to be seen (-0.486 RU). Resonance units are equivalent to change in wavelengths.

The P4SPR™ advantage

Affinité instruments' P4SPR™ is a very user-friendly instrument to perform general quality control of proteins. Whether a supply of proteins is from a new supplier or has been stored for a while, the P4SPR™ can easily distinguish between highly active and less active

ones. Additionally, antibody samples do not need much sample preparation and can be directly injected into the instrument. The P4SPR™ provides fast, real-time data and precision due to its multichannel feature. It is thus more advantageous than endpoint assays such as ELISA.

Conclusion

It is evident that a simple and portable SPR instrument can quickly establish which antibody source should be used for further experimentation due to its superior performance over others. This will certainly reduce a significant amount of lost time for researchers to disqualify less biologically active antibodies before conducting their experiments. Therefore, the P4SPR™ provides a quick and easy alternative to perform quality control on other types of biopharmaceutical products.

References

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About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built on deep knowledge accrued on over a decade of research in SPR. The commercialization of promising innovations is spearheaded by a diverse leadership experienced in business, science and engineering.

About the Author

Dr. April Wong is an analytical chemist and has research experience in the biosensor area for over a decade. She obtained her Ph.D. at the University of Toronto.