APPLICATION NOTE

Epitope characterization of influenza antiviral targets using OpenSPR™

Summary

Rapid changes in influenza antiviral target proteins due to antigenic drift result in cloaking of the influenza virus from the immune system of vaccinated hosts. Hence, annual formulation updates for influenza vaccines and related antibody therapies are required to preserve immune recognition against different influenza subtypes. As such, characterizing the binding kinetics and epitope diversity of various antibodies to influenza viral antigens is essential for treating and preventing potential outbreaks. In collaboration with Sino Biological, a global leader in recombinant technology, we use Nicoya's surface plasmon resonance (OpenSPR™) system to perform epitope characterization of several antibodies against Influenza A nucleoprotein (NP).

Introduction

Influenza, or "the flu", is a contagious viral infection caused by the influenza virus. Of the four types (Influenza A, B, C, and D), the two main flu viruses impacting humans are Influenza A and B, both being highly contagious and routinely causing seasonal epidemics. Furthermore, Influenza A viruses are the only known to cause flu pandemics, with all five historical instances of flu pandemics since 1900 being associated with Influenza A.

With the constant evolution of viruses, binding and characterization studies of viral antibodies and antigens are critical to managing and preventing future viral outbreaks. In addition, these studies advance our understanding of viral antigenic drift and diversity of flu-related antibodies.

Influenza A Nucleoprotein (NP)

Influenza viral nucleoprotein is a structural protein that plays a critical role in viral replication and host adaptation. Antibodies targeting NP proteins are commonly used for immunodetection of influenza viruses in various assays, including enzyme linked immunosorbent assays (ELISA), lateral flow assays (LFA), and direct fluorescent antibody tests. Due to the high frequency of antigenic drift or shift among different influenza strains, broad-spectrum influenza antibodies are particularly desired for flu diagnostics.

Traditional techniques such as ELISA and western blot (WB) require time-consuming washing and incubation steps, and depend on the use of tags for analysis. Compared to such traditional techniques, the information content provided by surface plasmon resonance (SPR) enables increased efficiency in the development of viral diagnostics and therapeutics. SPR provides complete kinetic characterization of antibody-antigen interactions to understand, rank, and optimize antibody therapeutics, without the use of tags or labels. In addition, it offers the unique advantage of epitope binning capabilities to better understand epitope diversity and overlap.

Epitope Characterization with OpenSPR

Kinetic characterization with OpenSPR enhances the study, development, and optimization of vaccines & antibody therapeutics, among many other applications, as it enables a deeper understanding of both antibody efficacy and antigen drift. Epitope binning is a competitive assay used to characterize the simultaneous binding of monoclonal antibodies (mAbs) to an antigen, tested in a pairwise manner, for determining whether they block one another's binding to the same epitope of the antigen. If the binding of one mAb to the antigen prevents the binding of another mAb, both mAbs can be clustered into groups (or bins) that all compete for the same or similar epitope. If both mAbs

are able to bind to the antigen, then they are considered to bind to distinct, non-overlapping epitopes.

There are three typical binning assays used: classical sandwich; tandem; and premix. In the classical sandwich assay, the first mAb is immobilized onto the sensor, followed by injection of the antigen and then the second mAb. In the tandem assay, the antigen is immobilized onto the sensor surface and the two mAbs are injected in sequence. Lastly, in the premix assay, the first mAb is immobilized onto the sensor, followed by injection of a solution of the antigen pre-incubated with an excess of the second mAb.

Sino Biological provides recombinant antigen products, including hemagglutinin (HA), neuraminidase (NA) and NP proteins, for all WHO-recommended vaccine strains, for use in SPR-based assays. In collaboration with Sino Biological, epitope characterization studies were conducted on OpenSPR in a classical binning format with five unique antibodies against an influenza A NP protein, with the results summarized in this application note.

Materials & Equipment

- OpenSPR-XT Instrument
- OpenSPR Carboxyl sensor (SEN-AU-100-10-HC-COOH)
- OpenSPR Carboxyl Reagent Kit (COOH-RK-10)
- 96-Well Plate (WP-10)
- TraceDrawer™ Kinetic Analysis Software
- Running Buffers: pH 7.4 PBS-T (0.05% Tween 20), PBS-T + 10 mM EDTA
- Regeneration Buffer: 10 mM Glycine-HCl pH 2.0
- Ligands and analytes: All viral products were supplied by Sino Biological. For a full list of products and their Catalog # please see Appendix.

Method

Pan Influenza A Nucleoprotein Antibody Binning

Following the start-up procedure in the software, the OpenSPR-XT instrument was primed, using PBS-T as the initial running buffer. A High Capacity Carboxyl Sensor was loaded into the instrument and the surface was prepared with an injection of 10 mM HCl in both channels at a flow rate of 150 µL/min.

Next, EDC/NHS solution from the OpenSPR™ Carboxyl Reagent Kit was used to activate the carboxyl surface in both channels at a flow rate of 20 µL/min. Each Pan Influenza A nucleoprotein antibody (100 nM in acetate pH 5.0) was directly immobilized on the sensor surface in both channels at a flow rate of 20 µL/min. Ethanolamine blocking solution was then used to deactivate the carboxyl surface in both channels at a flow rate of 20 µL/min. The instrument was buffer changed with PBS-T + 10 mM EDTA as the analysis running buffer.

PBS-T+10 mM EDTA was injected in both channels to collect blank injection curves, followed by the introduction of Influenza A NP antigen (50 nM) to each capture antibody in channel 2 only (designated as the sensing channel). Next, a Pan Influenza A nucleoprotein antibody was injected in both channels as the "secondary antibody" sample at 100 nM concentration. The sensor surface was then regenerated with 10 mM Glycine-HCl pH 2.0 at 150 µL/min to remove the secondary antibody and the antigen. This was repeated for each additional Pan Influenza A nucleoprotein antibody.

Lastly, the TraceDrawer™ Analysis Software was used to determine the RU response of the secondary antibody. No RU response of the secondary antibody indicates it was unable to bind to the captured antigen and competed for the same or similar epitope as the surface antibody.

Results & Discussion

Epitope Characterization

The epitope diversity of the Pan Influenza A nucleoprotein antibodies were evaluated with a classical sandwich assay. Figure 1 shows a representative plot of the antigen binding to the immobilized surface antibody, followed by either binding or no binding of the solution antibody to the antigen. Figure 2 shows the results of a 5x5 epitope bin with "Bind" results signifying a unique epitope targeted by the antibodies. The antigen binding responses were used to normalize the binding responses of the secondary antibody across all curves (since binding activity of the antigen to the surface can change over time). Analysis of secondary antibody binding was done by setting thresholds for the normalized solution antibody responses.

Bind, intermediate and not bind categories were defined as >30% of maximum response (Rmax), 10-30% of Rmax and <10% Rmax, respectively.

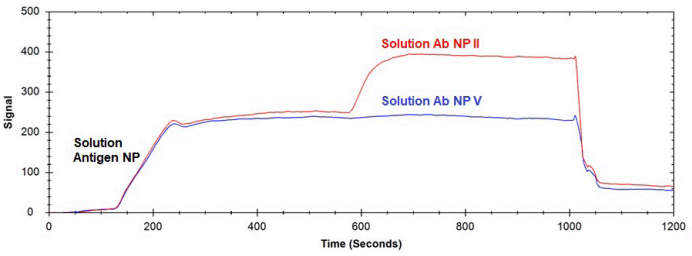

Figure 1: *Epitope characterization showing (normalized) capturing of the Influenza A NP Protein antigen to the immobilized surface antibody NP III followed by binding of the solution antibody to a different epitope of the antigen (Ab NP II). A lack of binding of the solution antibody NP V curve indicates epitope overlap of NP III with NP V. Regeneration is achieved by 10 mM glycine HCl pH 2.0.*

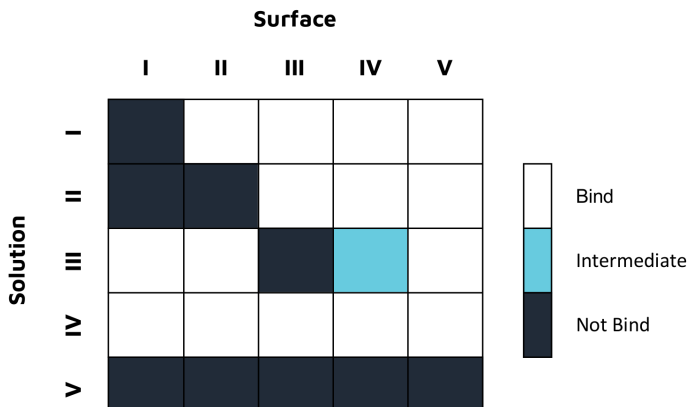

Figure 2: *Epitope characterization analysis for Pan Influenza A Nucleoprotein Antibody binding to Influenza A Nucleoprotein Protein (Cat: 40205-V08B). The degree of binding displayed in the heat map was determined based on the change in response levels after the addition of the solution antibody onto the surface antibody bound to the antigen. Bind, intermediate and not bind categories were defined based on above 30% Rmax, 10-30% Rmax and below 10% Rmax, respectively.*

Conclusion

OpenSPR was able to successfully characterize Sino Biological's highly specific influenza antibodies by conducting epitope analysis. Compared to traditional methods of characterization, SPR accelerates analysis of multiple interactions by eliminating the need for labels and reducing time-consuming preparation steps. This supports the use of the OpenSPR for applications in influenza diagnostics and vaccine development.

Appendix

Table 1: Sino Biological anti-NP antibodies (ligands).

	Name	Cat #
NP I	Pan Influenza A Nucleoprotein Antibody, Mouse MAb	40205-MM16
NP II	Pan Influenza A Nucleoprotein Antibody, Mouse MAb	40205-MM18
NP III	Pan Influenza A Nucleoprotein Antibody, Rabbit MAb	40205-R063
NP IV	Pan Influenza A Nucleoprotein Antibody, Rabbit MAb	40208-R014
NP V	Pan Influenza A Nucleoprotein Antibody, Rabbit MAb	40208-R017

Table 2: Sino Biological recombinant protein (analyte).

Name	Cat #
Influenza A H1N1 (A California/07/2009) Nucleoprotein / NP Protein (His Tag)	40205-V08B