Detection of Virus-Like Particles using OpenSPR-XT™

Summary

- Kinetic binding of a 10 MDa virus-like particle (VLP) to an immobilized monoclonal antibody (specific to the VLP) was measured using the OpenSPR-XT™ instrument.
- The VLP was detected in sub-nanomolar concentrations, establishing a high affinity between the VLP and the antibody.
- The OpenSPR-XT™ instrument can be used for the detection of very large molecules with an immobilized ligand.

Overview

Virus-like particles (VLPs) are complex molecules composed of multiple subunits that resemble a specific virus without its genetic material. VLPs are frequently used to study viruses without being exposed to their infectious components. Their applications include the development of therapies and vaccines against viral diseases, as well as the identification of viral protein components. In this application note, the OpenSPR-XT™ instrument was used to detect a VLP with an approximate molecular weight of 10 MDa to an immobilized antibody. Binding of the VLP could be detected at sub-nanomolar concentrations. The OpenSPR-XT™ is an affordable and user-friendly SPR instrument that enables the measurement of label-free binding kinetics of diverse biomolecular interactions.

Material and Equipment

- OpenSPR-XT™ instrument
- OpenSPR™ Protein A Sensor Kit
- Tracedrawer Kinetic Analysis Software
- Ligand: Monoclonal antibody, specific to the VLP
- Analyte: Virus-like particle (VLP)
- Running buffer: PBS-T (0.05% Tween-20) + 6.67% (w/v) sucrose, pH 7.0

Procedure

1. Following the start-up procedure in the software, the OpenSPR-XT™ instrument was set up, using PBS-T+sucrose as the running buffer.
2. The Protein A sensor surface was prepared following the steps outlined in the Protein A sensor kit and ligand immobilization wizard in the software.
3. The antibody was captured onto the Protein A surface with a flow rate of 10 µL/min, on channel 2 only (designated as the sensing channel).
4. The VLP sample (containing 40% sucrose) was diluted in PBS-T to a VLP concentration of 2 nM and sucrose concentration of 6.67% (w/v), and further diluted in 2-fold series in the running buffer.
5. The VLP analyte samples were analyzed in order of low to high concentration at 50 µL/min with an association period of 120 s and a dissociation period of 600 s.
6. The ligand was regenerated with a pH 1.5 Glycine-HCl injection at 100 µL/min between analyte injections.

Results & Discussion

Antibody capture using Protein A was chosen to ensure the antibody on the sensor surface was in an optimal orientation to bind to the VLP. Figure 1 shows the antibody capture of over 2000 RU onto the Protein A surface.

Figure 2 shows the binding of the VLP to the captured antibody at 4 different concentrations, with 2 nM as the highest concentration, and further analysis 2-fold serial dilutions down to 250 pM. Sucrose was included in the running buffer to aid in the stability of the VLP sample and to minimize buffer mismatch.

Since the VLPs are extremely large and composed of multiple subunits, the avidity of the system is unknown, therefore, in this case, a kinetic fit of the data would not be representative. However, since binding of the VLP could be clearly detected in sub-nanomolar concentrations, it can be determined that the antibody and VLP form a high-affinity system.

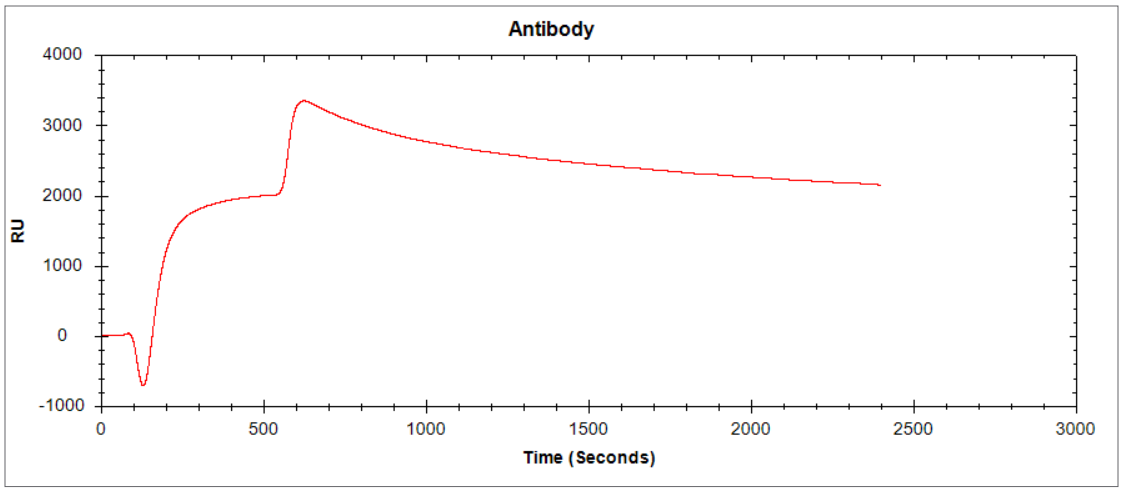

Figure 1: Ligand capture of the antibody to the Protein A sensor surface. The dip and jump at the start and end of the injection, respectively, is due to the bulk shift associated with the RI difference of running buffer and the antibody in sucrose-free PBS-T.

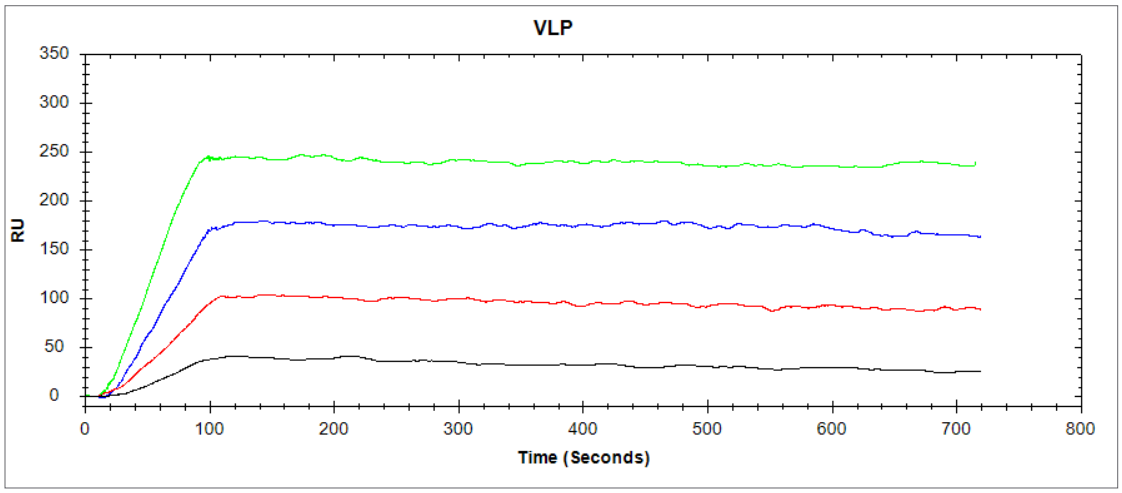

Figure 2: Binding of the VLP at 2000 pM, 1000 pM, 500 pM and 250 pM to the captured antibody.

Conclusions

This study demonstrates that the OpenSPR-XT™ can be used to detect binding between a captured antibody and an analyte with an approximate hydrodynamic radius of 40 nm and molecular weight on the order of several MDa. The detection of the VLP at sub-nanomolar concentrations establishes a high affinity between the VLP and the antibody. This supports the use of OpenSPR-XT™ for applications involving VLPs such as antibody screening for vaccine development.