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CoVALX
Solutions for Protein Complex Analysis

Application News

MALDI-2203

High Mass MALDI-TOF Mass Spectrometry

Characterization of intact mAb/SARS-CoV-2-S2 protein complexes by OmegaToF MS

■ Introduction

The applicability of MALDI-TOF mass spectrometry to perform protein detection is well recognized in the life science field. Using an OmegaToF MALDI mass spectrometer, it is now possible to analyze intact proteins and protein interactions up to 1500 kDa. This extended mass range allows the characterization of most non-covalent protein complexes, including the antigen/mAb complexes essential for the development of therapeutic proteins.

The identification of immunogenic epitopes in the SARS-CoV-2 spike (S) glycoprotein is essential for the advancement and development of new therapeutics against COVID-19. The spike glycoprotein is expressed on the exterior of the SARS-CoV-2 virion and contains two subunits, S1 and S2. These subunits are key elements mediating viral entry and infection. These subunits are also antigenic determinants stimulating the immune response against the virus. In comparison with S1, the S2 subunit is highly conserved and the target of many new therapeutic monoclonal antibodies.

Here, we demonstrate the capability of the OmegaToF mass spectrometer (OmegaTOF, Fig. 1) and its extended mass range to perform high-mass detection of anti-SARS-CoV-2-S2/SARS-CoV-2-S2 (Fig 2) intact protein complexes.

Figure 1: OmegaToF MALDI Mass Spectrometer

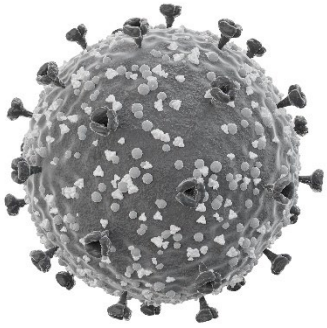

Figure 2: Spike protein from SARS-CoV.

■ Sample Preparation and Measurement Conditions

The samples – SARS-CoV-2-S2 and the monoclonal antibody anti-SARS-CoV-2-S2 – were prepared at a concentration of 2pmol/µL and 1pmol/µL, respectively. Fifty laser shots were accumulated per profile (200 profiles per spectrum). The mass spectra were recorded using the average masses.

For cross-link experiments, 9µL of the antibody/antigen sample prepared previously was mixed with 1µL of R200 cross-linking reagent, also produced by CovalX, to reach a final concentration of 1mg/mL. The sample was incubated at room temperature for 180 minutes before mass spectrometric analysis.

■ Results

To demonstrate the sensitivity of the OmegaToF, the spectra of the intact non-covalent protein complexes produced by SARS-CoV-2-S2/anti-SARS-CoV-2-S2 are presented.

Fig. 3 shows the mass spectrum of the mixture. The singly-charged ions (approx. 148kDa) of the anti-SARS-CoV-2-S2 monoclonal antibody and the singly-charged ions (approx. 87kDa) of SARS-CoV-2-S2 antigen were observed with good signal-to-noise ratio.

Fig. 4. shows the mass spectrum of the same mixture SARS-CoV-2-S2/anti-SARS-CoV-2-S2 which was analyzed in Fig. 1 after cross-linking. The singly-charged ion of the SARS-CoV-2-S2/anti-SARS-CoV-2-S2 complex (approx. 343kDa, stoichiometry 2:1) and the singly-charged ion of SARS-CoV-2-S2 (approx. 93kDa) were observed. The singly-charged ion of the anti SARS-CoV-2-S2 antibody observed in Fig.1 is difficult to observe (approx. 153 kDa) due to the binding with SARS-CoV-2-S2.

Fig. 5 shows an overlay of the control (Fig. 3) and cross-link (Fig. 5) spectra. The singly-charged ion (approx. 326kDa) of the SARS-CoV-2-S2/anti-SARS-CoV-2-S2 complex with stoichiometry 2:1 is observed. The stoichiometry 1:1 is not observed for this experiment and this sample due to the high affinity of the monoclonal antibody and the excess of antigen.

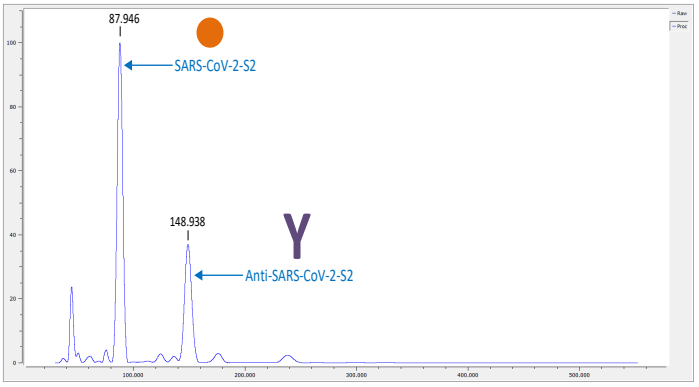

Figure 3: OmegaToF spectrum of SARS-CoV-2-S2/Anti-SARS-CoV-2-S2

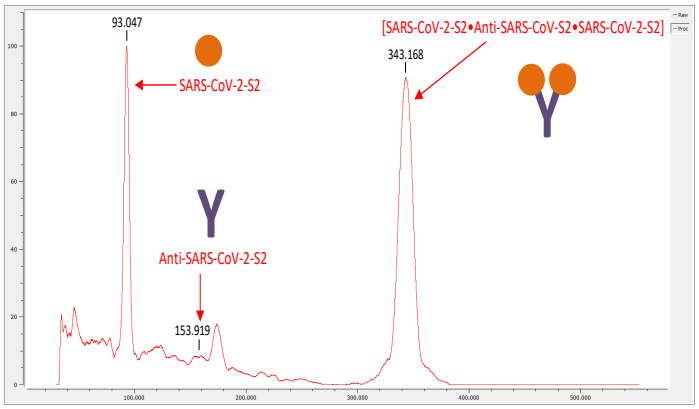

Figure 4: Detection of SARS-CoV-2-S2/Anti-SARS-CoV-2-S2 protein complexes after cross-linking

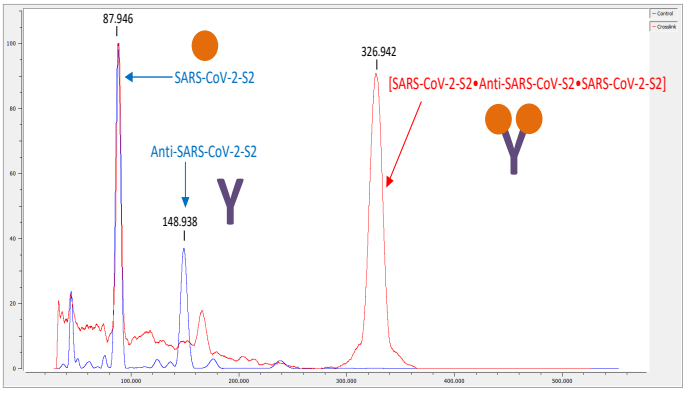

Figure 5: OmegaToF overlay spectrum (control, blue; cross-link, red) of the SARS-CoV-2-S2/Anti-SARS-CoV-2-S2 protein complexes

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