SHIMADZU
Excellence in Science

COVALX
Solutions for Protein Complex Analysis

Application News

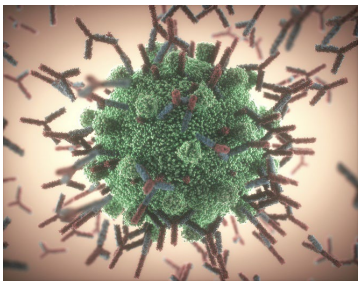
MALDI-2201

High Mass MALDI-TOF Mass Spectrometry

Characterization of intact monoclonal Antibody/Antigen complexes by the OmegaToF MS

■ Introduction

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



The extended mass range of the OmegaToF gives direct access to the characterization of complexes formed between therapeutic monoclonal antibodies (mAbs) and their antigen targets. The analysis becomes routine as the samples are analyzed directly in their original buffers, with the sensitivity and robustness of standard MALDI ToF mass spectrometric analysis.

Here, we demonstrate the capability of the OmegaToF mass spectrometer (OmegaTOF, Fig. 1) to perform high-mass detection of intact antigen/mAb protein complexes.

Figure 1: OmegaToF MALDI Mass Spectrometer

■ Sample Preparation and Measurement Conditions

Antigen and antibody samples were purchased from Sino Biological. These were prepared at a final concentration of 1pmol/µL each in the final mixture. Fifty 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 is mixed with 1µL of R200 cross-linking reagent [CovalX R200] to reach a final concentration of 1mg/mL. The sample is incubated 180 minutes before direct mass spectrometric analysis in the OmegaToF.

■ Antibody : Antigen Complex

To demonstrate the OmegaTOF sensitivity, the antigen/antibody complex spectra are presented. Fig. 3 shows the mass spectrum of the antigen/antibody mixture. The singly-charged ion of the antigen (approx. 107kDa) and the singly-charged ion of the antibody (approx. 143kDa) were observed with good signal-to-noise ratio.

Fig. 4 shows the mass spectrum of the same antigen/antibody mixture analyzed in Fig. 3 after cross-linking. The singly-charged ion of the antigen/Antibody complex (approx. 258kDa, stoichiometry 1:1) and the singly-charged ion of the antibody (approx. 148kDa) were observed. The singly-charged ion of the antigen observed in Fig. 3 is not observed after crosslinking due to the antibody binding.

Fig. 5 shows an overlay of the control (Fig. 3) and cross-link spectra (Fig. 4). The singly-charged ion (approx. 248 kDa) of the antigen/antibody complex with stoichiometry 1:1 is clearly observed as the primary signal after crosslinking. The stoichiometry of 2:1 is not observed for this experiment.

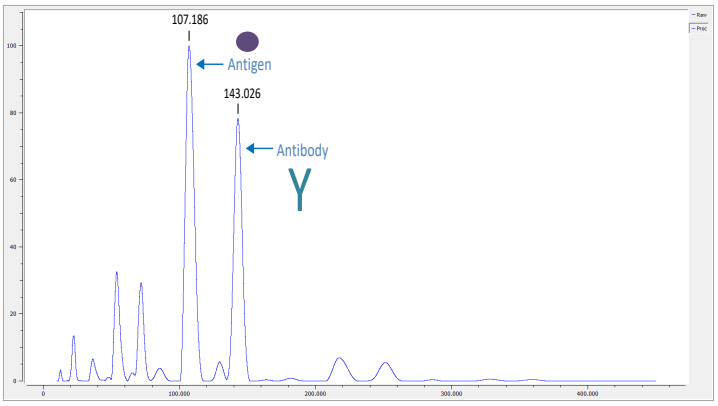


Figure 3: OmegaToF spectrum of antibody/antigen mixture.

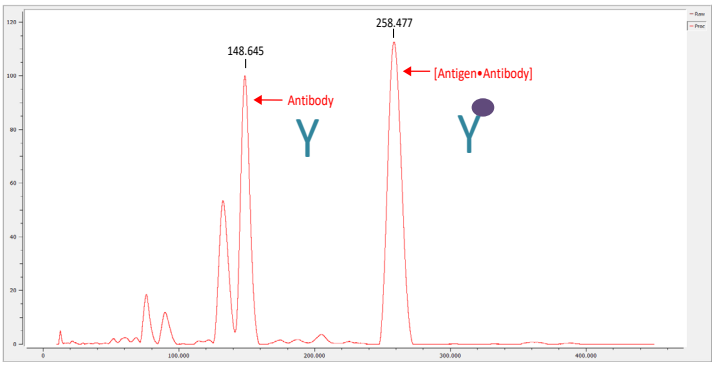


Figure 4: Detection of antibody-antigen complex after cross-linking.

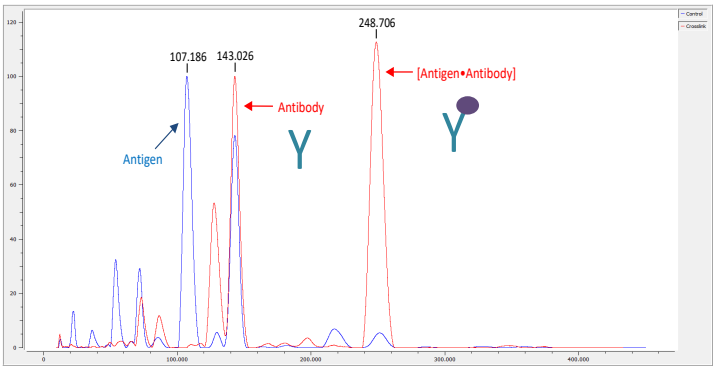


Figure 5: Overlay (control, blue; cross-link, red) of antibody-antigen complex.

■ Two Antibodies Binding One Antigen

This technology can also be used to characterize whether two different monoclonal antibodies are able to bind onto the antigen protein at the same time. If they are able to bind at the same time, then this signifies that they are binding onto different epitope regions of the antigen protein.

Fig. 6 shows an overlay of two mass spectra containing two different monoclonal antibodies against the same target antigen protein. In blue is the control spectra of the two monoclonal antibodies and the antigen mixed together and analyzed together. In the blue (control) spectrum, the singly-charged ions of Antibody 1 or Antibody 2 (approx. 147 kDa) and the singly charged ion of the Antigen (approx. 87 kDa) were observed.

The red spectrum is the signal of the crosslinked material from the same control (blue) sample. The detection of a complex binding both monoclonal antibodies (approx 384kDa) and the antigen together at the same time provides insight that the two monoclonal antibodies are binding different epitope regions on the antigen protein.

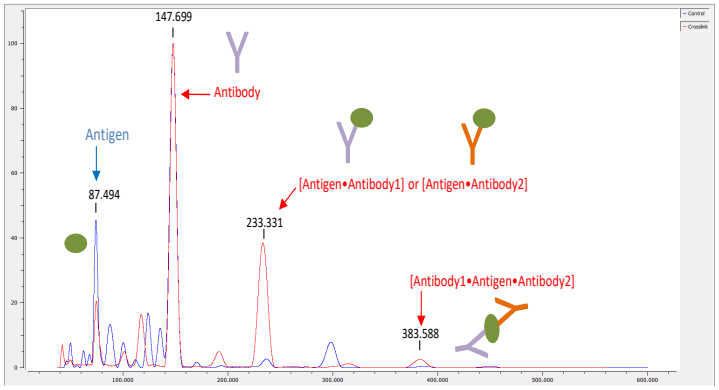


Figure 6: Overlay (control, blue; crosslink, red) of mixture containing two different monoclonal antibodies (Antibody 1 and Antibody 2) and target antigen. `

First Edition: February 2022

SHIMADZU Corporation
www.shimadzu.com/an/

SHIMADZU SCIENTIFIC INSTRUMENTS
7102 Riverwood Drive, Columbia, MD 21046, USA
Phone: 800-477-1227/410-381-1227, Fax: 410-381-1222
URL: www.ssi.shimadzu.com