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Application News

MALDI-2202

High Mass MALDI-TOF Mass Spectrometry

Detection of Ultra High-Mass Proteins Using the OmegaToF Mass Spectrometer

■ Introduction

The ability of MALDI-TOF mass spectrometry to sensitively detect a protein's mass is well recognized in the life science field. Using MALDI ToF MS, the molecular weight of proteins is determined with high accuracy and sensitivity when compared with non-mass spectrometric methods. However, the analysis of ultra-high molecular weight proteins (≥400 kDa) is still difficult to achieve using standard MALDI ToF instrumentation. Due to the high mass detection capabilities of the OmegaToF, analyzing high molecular weight proteins becomes routine.

The extended mass range of the OmegaToF gives full access to macromolecules up to 1500 kDa with nanoMolar sensitivity. The analysis of ultra high mass molecules such as IgM or KLH becomes possible within seconds, increasing speed and accuracy of quality control and development processes for these therapeutic proteins. OmegaToF mass spectrometry offers a larger mass range for fast and reliable analysis of protein biotherapeutics.

This application note demonstrates the capability of the OmegaToF mass spectrometer (OmegaTOF, Fig. 1) to perform high-mass detection of IgM (Fig 2) and KLH (Fig. 3).

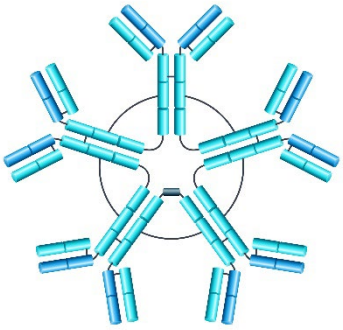

Figure 2: Generalized structure of an IgM Antibody

■ Sample Preparation and Measurement Conditions

The samples of Immunoglobulin M (IgM) and Keyhole Limpet Hemocyanin (KLH) were purchased from Sigma-Aldrich. These were prepared at a concentration of 300nmol/µL and 250nmol/µL, respectively. Fifty shots were accumulated per profile (200 profiles per spectrum). The mass spectra were recorded using the average masses.

Figure 1: OmegaToF benchtop MALDI ToF MS

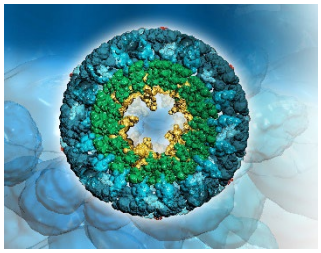

Figure 3: CryoEM reconstruction of Keyhole Limpet Hemocyanin (KLH)

■ Results

To demonstrate the OmegaTOF's sensitivity for ultra high-mass macromolecules, the spectra of IgM and KLH are presented. Fig. 4 shows the mass spectrum of IgM. The singly- (approx. 971kDa), doubly- (approx. 486kDa), triply-charged (approx. 325kDa) and quadruply-charged ions (approx. 243kDa) were observed with good signal-to-noise ratio. IgM ions of this mass are not detected with conventional MALDI-TOF instrumentation.

Fig. 5 shows the mass spectrum of the KLH protein. The singly- (approx. 467kDa), doubly- (approx. 238kDa) charged ions were observed with good signal-to-noise ratio. Other ions were observed with approx. 121kDa, 178Kda, 295kDa, 315kDa, 345kDa and 402kDa.

These example spectra demonstrate the ability to detect high mass ions containing multiple ion species over a broad mass range. This direct and rapid analysis can be utilized routinely in the development of protein biotherapeutics.

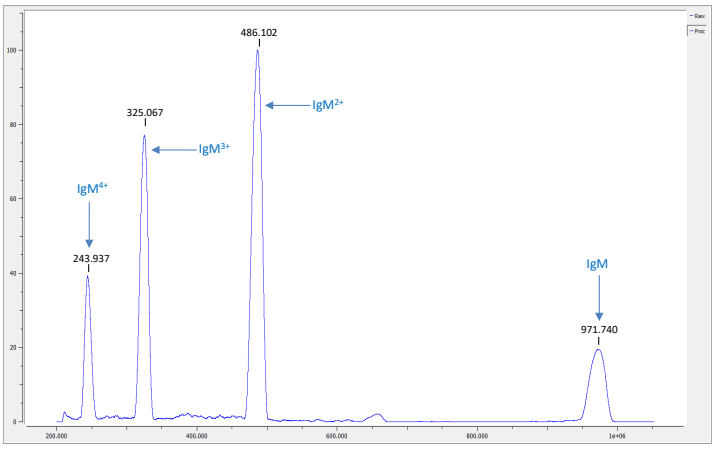

Figure 4: OmegaToF spectrum of IgM

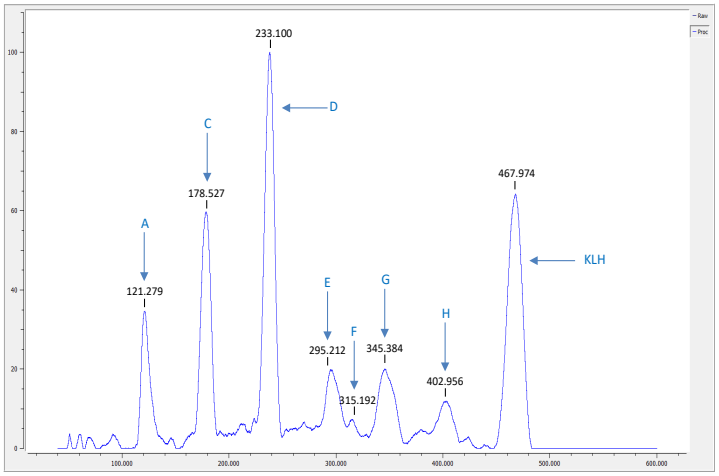

Figure 5: OmegaToF spectrum of KLH

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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