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Application Data Sheet

No. MALDI-003

MALDI-TOF
MALDI-TOF Mass Spectrometry

Analysis of Antibody Aggregates Using the AXIMA MegaTOF™ High Mass MALDI-TOF Mass Spectrometer

Introduction: Antibody aggregation remains a complex question to address in the development and manufacture of therapeutic antibodies and biosimilars. This primary degradation product can lead to several undesirable consequences such as immunological response and decreased efficacy. Current technologies for analyzing aggregation products include size exclusion chromatography (SEC), light scattering and analytical ultracentrifugation; however each of these techniques have limitations. As a result, regulatory agencies such as the FDA are leaning towards requesting complimentary data to improve characterization of therapeutic proteins[1, 2]. Recent developments in High Mass Matrix Assisted Laser Desorption Time of Flight Mass Spectrometry (HM MALDI-TOF MS) can fill these gaps by providing higher resolution data with improved mass accuracy to a mass range up to > 1.5MDa.

This technical note illustrates the ability to characterize antibody aggregates by covalently stabilizing them with cross-linking reagents, fractionating the cross-linked aggregates by SEC and finally analyzing the fractions using the AXIMA MegaTOF.

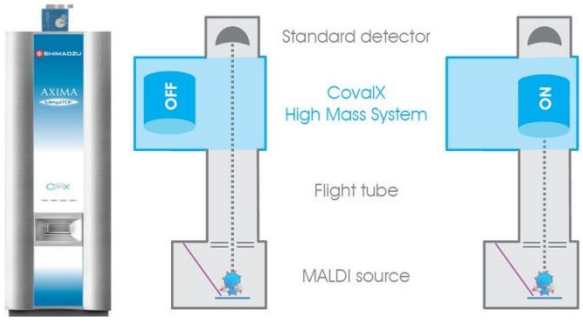

Figure 1 (above): The AXIMA MegaTOF integrates the high mass measuring capabilities of the CovalX detector (CovalX, Zurich, Switzerland) with Shimadzu's AXIMA MALDI-TOF MS. The MegaTOF provides users the flexibility to move the CovalX detector in and out of position to measure across the full mass range (up to 1.5 MDa).

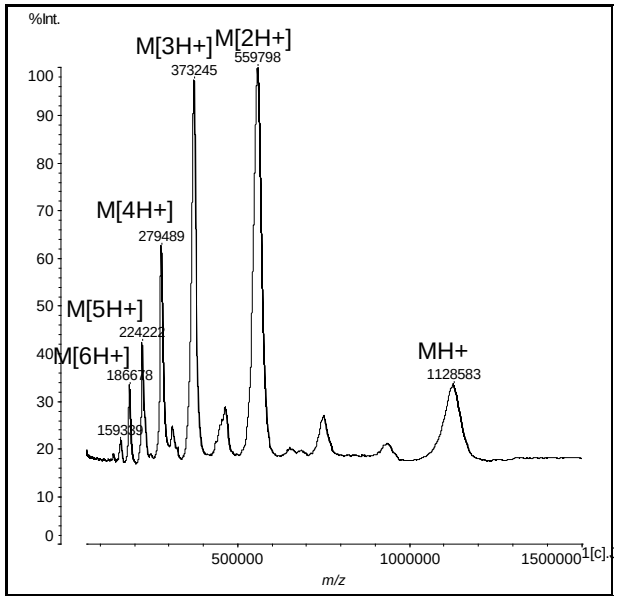

Figure 2 (right): Successful measurement of IgM at 1.1MDa using the AXIMA MegaTOF.

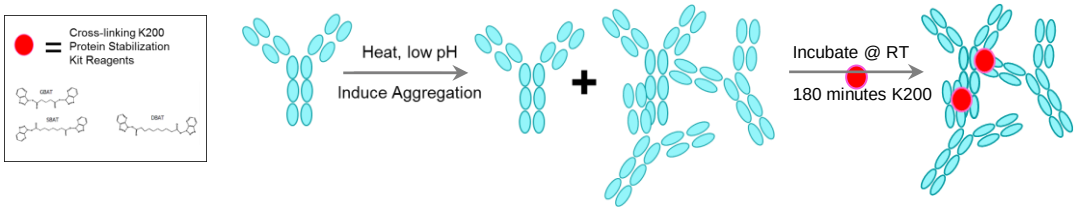

Figure 3: Traditionally, the MALDI sample preparation and/or ionization process causes non-covalently linked protein aggregates to dissociate. Because of this dissociation, it is necessary to stabilize the aggregates using cross-linking reagents that covalently bind proteins in close proximity (4-16Å) with each other. The CovalX K200 Stabilization kit provides a cocktail of multiple cross-linking reagents at different lengths to more effectively stabilize the aggregates. Once stabilized, the aggregates can be analyzed using the AXIMA MegaTOF.

For more information on the AXIMA MegaTOF, please visit: **www.megatof.com**

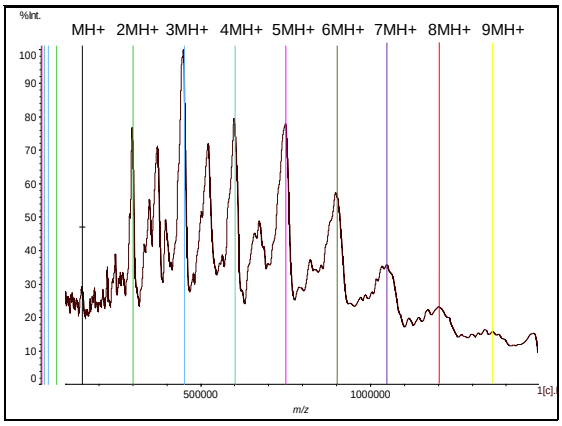

Figure 4a: The stabilized aggregate sample was separated by SEC and two fractions were collected: high mass aggregates and low mass monomer. The high mass fraction (shown here) successful stabilization of the antibody aggregates and detection out to a nonamer (9MH+) at 1.35 MDa.

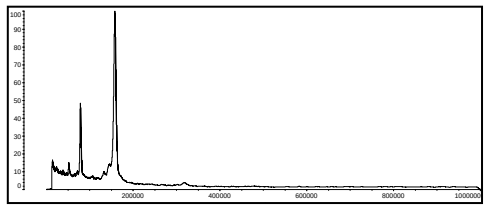

Figure 4b: In order to monitor for any undesirable plume aggregate formation, it is necessary to run a control sample that has not been cross-linked. Here only monomer peaks are detected, to verify that the aggregates seen in 4a were not due to adverse MALDI plume formation.

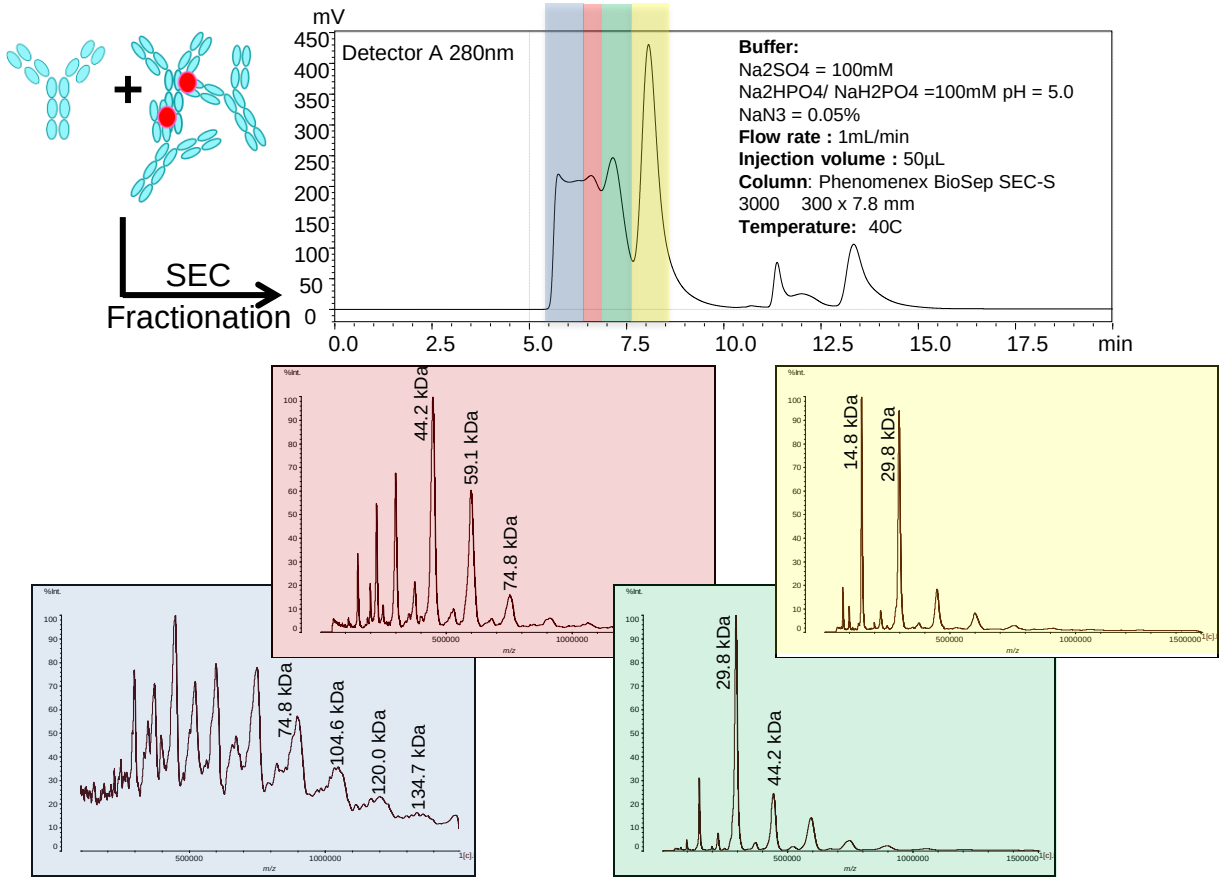

Figure 5 (above): SEC of the stabilized, stressed antibody sample was performed to fractionate specific peaks of the chromatogram. HM MALDI-TOF spectra indicate that the collected fractions separate the aggregates as expected with many of the higher order aggregates eluting in the blue fraction, the trimer, tetramer and pentamer eluting in the red fraction, the dimer in the green fraction and the monomer in the yellow fraction.

Results and Discussion: HM MALDI-TOF MS analysis of antibody aggregates is now possible when samples have been stabilized through the use of chemical cross-linking reagents such as the K200 kit from CovalX. The capability of the AXIMA MegaTOF to effectively detect ions up to 1.5 MDa provides the necessary instrumentation to effectively measure soluble, lower order aggregates. The AXIMA MegaTOF strategy for antibody aggregate analysis provides an alternate technology to accurately visualize - based on mass - what is present within the different fractions eluting from the SEC. Besides being rapid, accurate and sensitive, this method provides a direct analysis with much higher resolution then any of the existing technologies.

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