# High Mass MALDI-TOF MS Strategy for Antibody Aggregate Characterization

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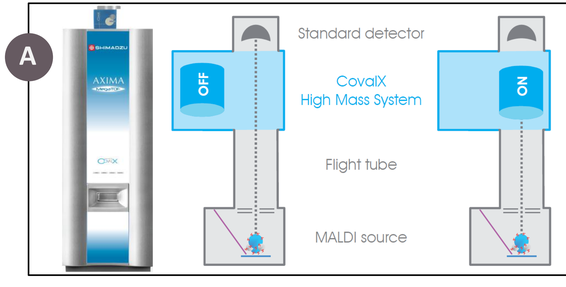
## Introduction

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 of 1.5 – 2.0 MDa. This presentation focuses on illustrating the capabilities of an integrated HM MALDI-TOF MS, the MegaTOF which integrates a standard AXIMA MALDI-TOF MS (Shimadzu, Kyoto Japan) with the high mass detection capabilities of the CovalX detector (CovalX, Zurich, Switzerland)

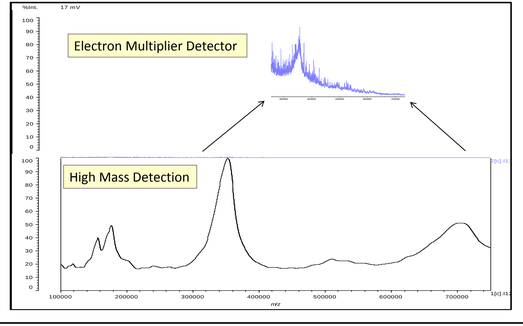
## Background

A




**HM MALDI Configuration:** The HM MALDI-TOF MS is a fully integrated system that incorporates the high mass detection capabilities of the high mass detector (CovalX Zurich, Switzerland) with a MALDI-TOF MS (Shimadzu Kyoto, Japan). Because the CovalX detector provides increased sensitivity and resolution at MH+ > 100,000 Da, the high mass detector is inter-changeable with the standard linear electron multiplier detector.

B

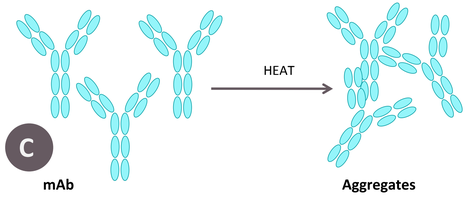



**High Mass Detection:**

The MegaTOF™ improves sensitivity and resolution of MH+ > ~100 kDa. The image at right shows the detection of thyroglobulin (~700kDa) using the electron multiplier detector (blue) and the CovalX detector (black). The standard linear detector has been scaled to the intensity of the CovalX detector.

## Therapeutic Protein Aggregation

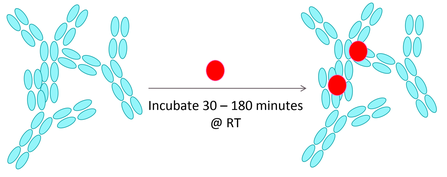
C

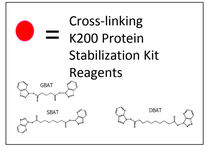



**Protein Aggregation Mechanisms:** Although not completely understood, several conditions during manufacture and storage can contribute to protein aggregation. These include high temperatures, acidic conditions, shear and presence of contaminants. In this experiment, proteins were heated to 50°C for 10 minutes.

## Crosslinking Chemistry

D



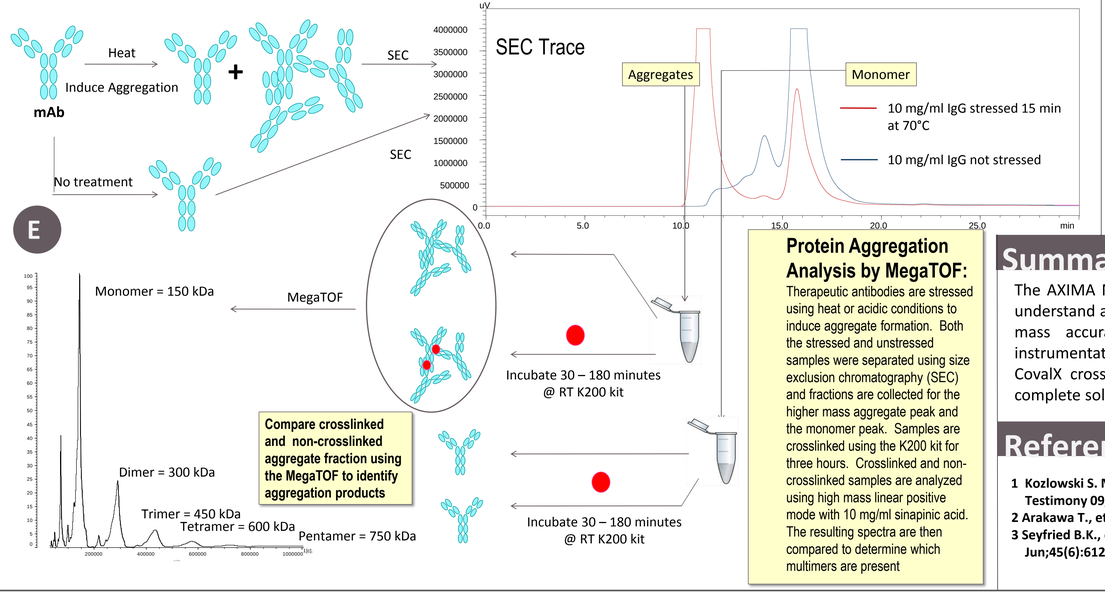




**Protein Crosslinking (Above):** During MALDI-TOF MS analysis, aggregates will dissociate. As a result, it is necessary to stabilize these complexes prior to ionization. The K-200 crosslinking reagent kit (CovalX, Zurich Switzerland) reacts with amine groups to covalently bind proximal amino acids.

**Protein Crosslinking (Below):** Serial dilutions of Glutathione Serine Transferase (GST) were made (1, ½, ¼, 1/8, 1/16) into purification buffer (CovalX, Zurich Switzerland). 1 µl K200 crosslinking reagent was added to 9 µl of each dilution to crosslink. Samples were analyzed in high mass linear positive mode with 10 mg/ml sinapinic acid (Sigma, Saint Louis, MO).

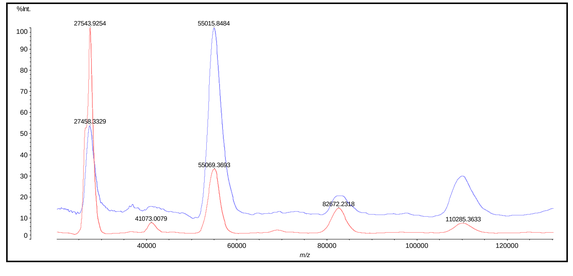
## MegaTOF Aggregation Workflow

E




**Compare crosslinked and non-crosslinked aggregate fraction using the MegaTOF to identify aggregation products**

**Protein Aggregation Analysis by MegaTOF:** Therapeutic antibodies are stressed using heat or acidic conditions to induce aggregate formation. Both the stressed and unstressed samples were separated using size exclusion chromatography (SEC) and fractions are collected for the higher mass aggregate peak and the monomer peak. Samples are crosslinked using the K200 kit for three hours. Crosslinked and non-crosslinked samples are analyzed using high mass linear positive mode with 10 mg/ml sinapinic acid. The resulting spectra are then compared to determine which multimers are present




## Summary

The AXIMA MegaTOF provides new opportunities to biopharmaceutical researchers to better understand and characterize protein aggregation. This HM MALDI-TOF MS system extends the mass accuracy, resolution, speed and user friendly interface provided my MALDI instrumentation to a mass range previously unavailble with this technique. Combined with CovalX cross-linking reagents and the Complex Tracker Software, the MegaTOF provides a complete solution for identifying aggregation products up to 1.5 MDa – 2.0 MDa.

## References

1 Kozlowski S. M.D. *Potential Need for Measurement Standards to Facilitate R&D of Biological Drugs.* Congressional Testimony 09/24/2009 -http://www.fda.gov/NewsEvents/Testimony/ucm183596.htm

2 Arakawa T., et al., *Aggregation Analysis of Therapeutic Proteins Part 1.* Bioprocess International, 2006, 4(10): 42-43.

3 Seyfried B.K., et al., *MALDI linear TOF mass spectrometry of PEGylated (glyco)proteins.* J Mass Spectrom. 2010 Jun;45(6):612-7.