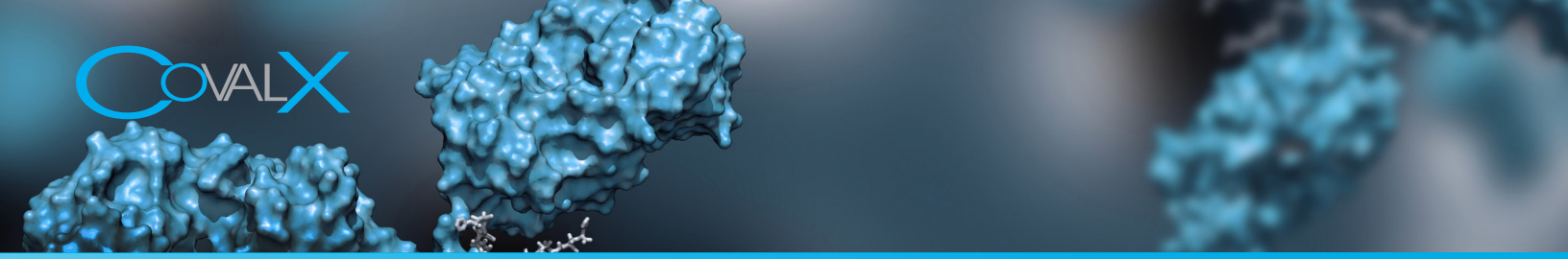


Interaction Analysis by High-Mass MALDI Mass Spectrometry

Introduction

CovalX is the solution provider for fast, sensitive and accurate analysis of protein interactions by MALDI mass spectrometry. Unfragmented and undigested, the protein complexes are detected intact using a specially developed High-Mass detection system. With no need for immobilization, buffer exchange or special tags, CovalX's solution allows the characterization of protein complexes, antibodies or therapeutic protein aggregates directly in the relevant buffer or formulation.

CovalX's technology analyzes protein interactions in three steps (Figure 1):

1. Specially developed cross-linking reagents and protocols stabilize the non-covalent interactions
2. High-Mass MALDI ToF MS analysis directly the intact protein complex
3. Post-acquisition data analysis software efficiently evaluates the data generated

Providing qualitative and quantitative data on:

- Protein complexes: Interaction validation, Complex stoichiometry, effect of compounds on protein interactions
- Antibody characterization: Antigen binding, Epitope mapping, Multibinding assays
- Therapeutic protein aggregation: Characterization, Semi-quantification
- PEG-protein Characterization

MALDI MS Analysis Kit

CovalX's different MALDI Stabilization Kits stabilize non-covalent protein interactions for analysis by MALDI mass spectrometry. The stabilizing reagents "freeze" covalently the protein interactions directly in the relevant sample matrix such as a pharmaceutical formulation. With no need for buffer exchange or dilutions, you can now characterize interactions in the buffer that is relevant for you.

High-Mass Detector Retrofit System

CovalX's High-Mass systems have been developed for every standard MALDI ToF instrument, allowing the analysis of macromolecules up to 2 MegaDaltons with outstanding nanoMolar sensitivity. Maintain the standard performance of your MALDI ToF instrument and add a second high-mass detector without removing anything.

CovalX Crosslinking Consumables

CovalX High-Mass Detector System

Complex Tracker Analysis Software

The latest release of CovalX's Complex Tracker Softwares provides a user-friendly interface for the post-acquisition automated detection and evaluation of mass spectrometric data generated by High-Mass MALDI ToF MS experiments.

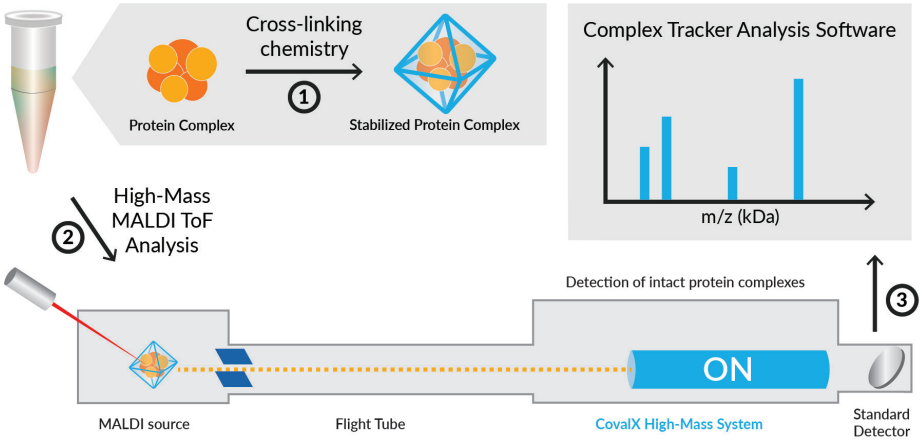


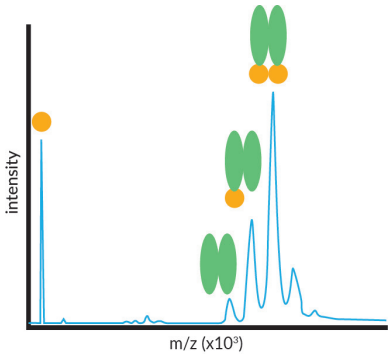
Figure 1
Interaction Analysis by High-Mass MALDI ToF MS

Step 1. Specially developed cross-linking reagents and protocols stabilize the non-covalent interactions
Step 2. High-Mass MALDI ToF MS analysis directly detects the complex intact
Step 3. Post-acquisition data analysis software efficiently evaluates the data generated

Interaction Analysis by High-Mass MALDI Mass Spectrometry

Protein Complex Analysis

Using cross-linking chemistry and High-Mass MALDI ToF mass spectrometry direct analysis of non-covalent protein complexes becomes possible. In Spectrum A, chemical cross-linking combined with high-mass matrix-assisted laser/desorption ionization mass spectrometry was used as a fast screening technique to determine binding specificities of intact nanobody-membrane protein complexes. Additionally, a titration series were performed to rank the binding affinity of the interacting nanobodies with CovalX's K200 MALDI MS analysis kit. The non-covalent complexes corresponding to the stoichiometry [2Nanobodies + 2Membrane proteins] are easily detected using the CovalX High-Mass MALDI system.



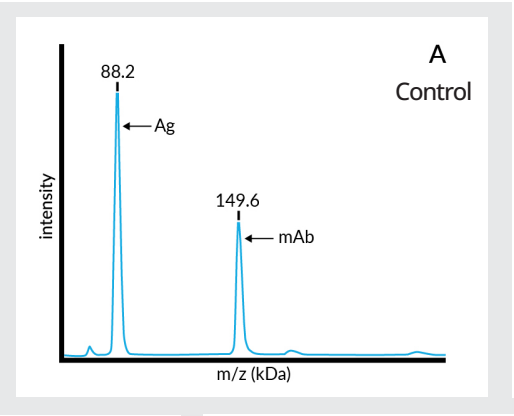
Kohler, M, et. al, Binding Specificities of Nanobody•Membrane Protein Complexes Obtained from Chemical Cross-Linking and High-Mass MALDI Mass Spectrometry Anal. Chem. 2018, 90, 8, 5306–5313

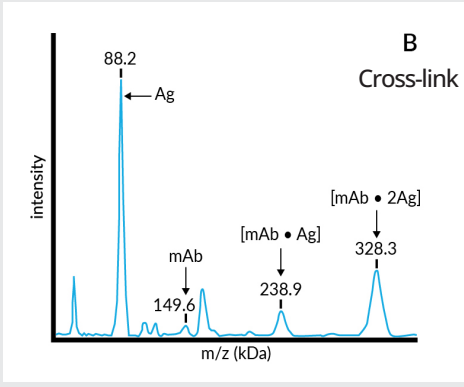
Therapeutic Protein Aggregation

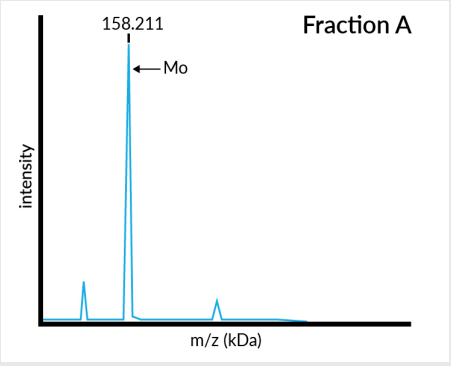
The analysis of the aggregation phenomenon of therapeutic proteins is of crucial importance as more and more pharmaceutical products are proteins. CovalX introduces a unique tool for the direct characterization of therapeutic protein samples. High-mass MALDI-TOF MS analysis different fractions containing recombinant IgA1 purified from the culture supernatant of cells are shown. (A) One main peak (158 kDa) corresponding to monomer (Mo) was detected in the fraction A. (B) Two main peaks (206 and 408 kDa) corresponding to a di-cation dimer (Di^{2+}) and a dimer (Di) were detected in fraction B. (C) Three main peaks (361, 561 and 717 kDa) corresponding to a di-cation tetramer (Te^{2+}), trimer (Tr), and tetramer (Te) were detected in fraction C.

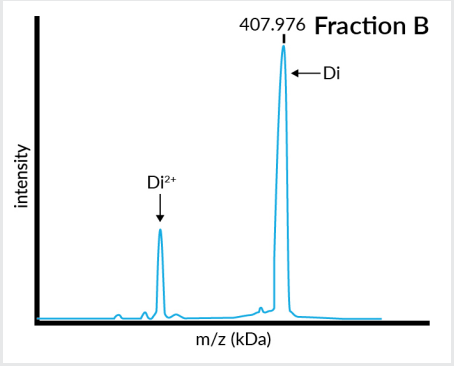
Antibody Characterization

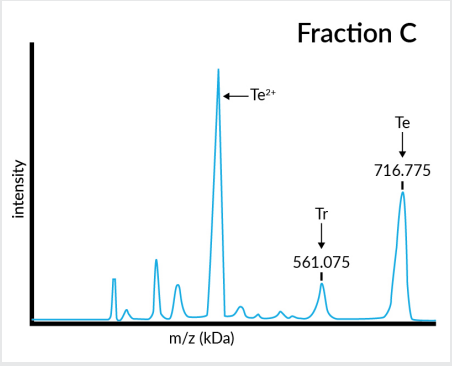
The characterization of antibodies including antigen binding assays, epitope mapping and multibinding assays, is performed directly using the CovalX technology. In Figure A (Control) and B (Cross-link), the protein complexes formed by the target antigen protein (88.2 kDa) and a monoclonal antibody (149.6 kDa) are detected before cross-linking (A) and after cross-linking (B) with CovalX's K200 MALDI MS analysis kit. After cross-linking, the non-covalent complexes [mAb • Ag] and [mAb • 2Ag] are easily detected with 238.9 and 328.3 kDa using the CovalX High-Mass system.











Saito S, et al. (2019) IgA tetramerization improves target breadth but not peak potency of functionality of anti-influenza virus broadly neutralizing antibody. PLoS Pathog 15(1): e1007427

CovalX
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