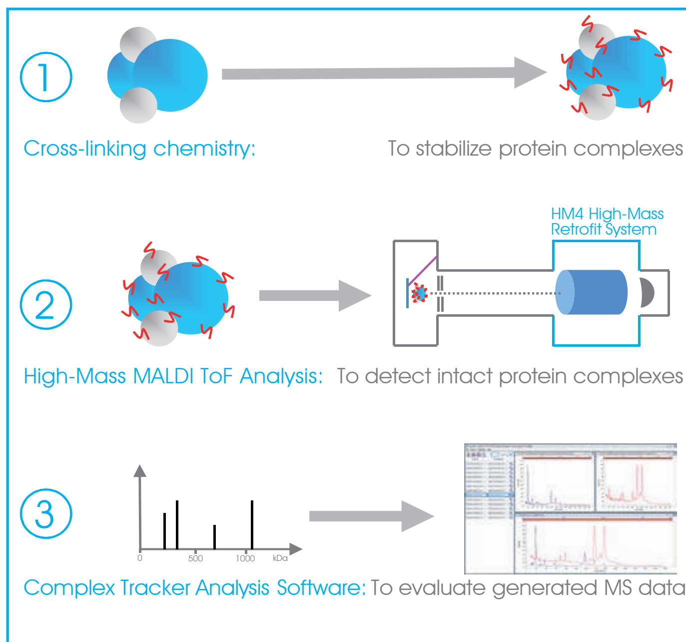
Interaction Analysis by High-Mass MALDI Mass Spectrometry

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:

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



Interaction Analysis by High-Mass MALDI ToF MS

K200 MALDI MS Analysis Kit

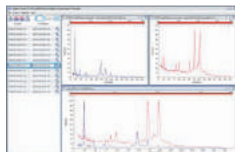
MALDI MS Analysis Kit:

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 from 5kDa to beyond 2MDa with outstanding nM sensitivity. Maintain the standard performance of your MALDI ToF instrument and add a second high mass detector.

HM4 or Pearl High-Mass Systems



Complex Tracker 2.0

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.

Providing Qualitative and Quantitative data on:

- **Protein complexes: Interaction validation, Complex stoichiometry, Interaction inhibition**
- **Antibody characterization: Antigen binding, Epitope mapping, Multibinding assays**
- **Therapeutic protein aggregation: Characterization, Semi-quantification**
- **PEG-protein Characterization**

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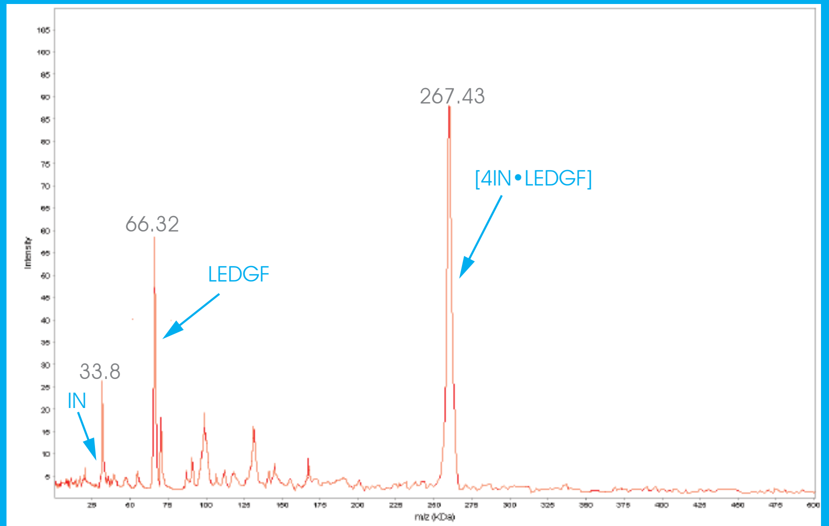
CovalX

Protein Complex Analysis

Using cross-linking chemistry and High-Mass MALDI ToF mass spectrometry easy analysis of non-covalent protein complexes becomes possible. In the spectrum presented, the protein complexes formed by HIV Integrase (IN) and cofactor LEDGF are characterized after cross-linking with CovalX's K200 MALDI MS analysis kit. The non-covalent complexes corresponding to the stoichiometry [4IN•2LEDGF] are easily detected using the CovalX High-Mass MALDI system.

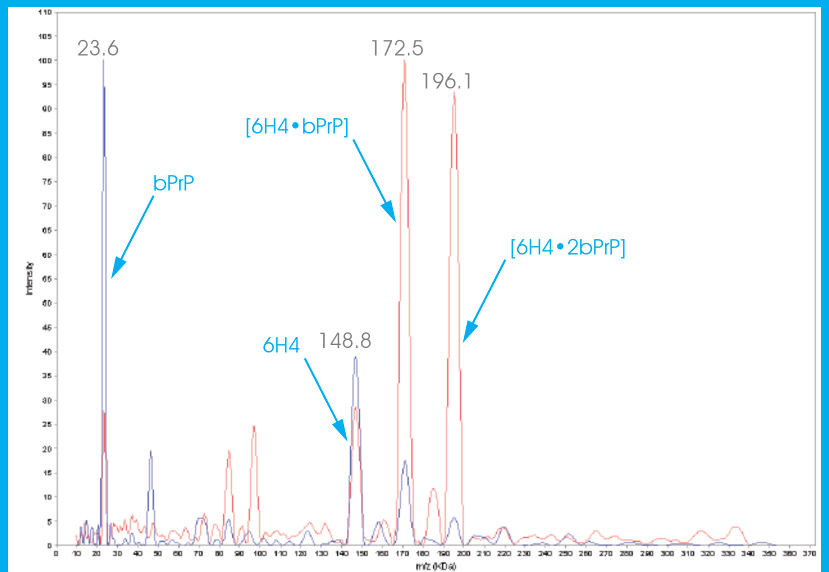
(Michel et al., The EMBO Journal (2009) 28, 980 - 991).



Antibody Characterization

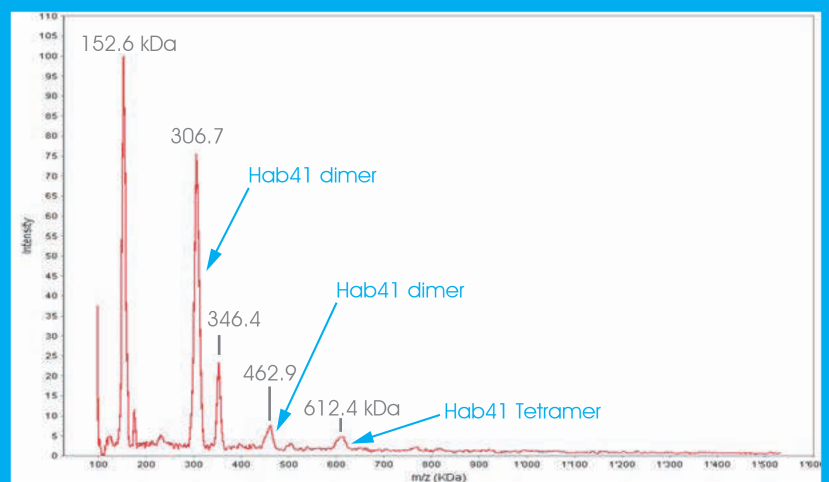
The characterization of antibodies including antigen binding assays, epitope mapping and multibinding assays, is easy using the CovalX technology. In the spectrum presented, the protein complexes formed by the bovine prion protein (bPrP) and a monoclonal antibody anti-bPrP (6H4) are detected before cross-linking (blue) and after cross-linking (red) with CovalX's K200 MALDI MS analysis kit. After cross-linking, the non-covalent complexes [6H4•bPrP] and [6H4•2bPrP] are easily detected with 172.5 and 196.1 kDa using the CovalX High-Mass system.

(Nazabal et al., Anal. Chem. (2006) 78, 3562-3570).



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. In the spectrum presented, an aggregated sample of a therapeutic antibody (Hab41) has been subjected to Size Exclusion Chromatography followed by a cross-linking protocol and high-mass MALDI ToF mass spectrometry. The spectrum obtained from the fraction analyzed shows the presence of a dimer (306 kDa), a trimer (462 kDa) and a tetramer (612 kDa) of Hab41.



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