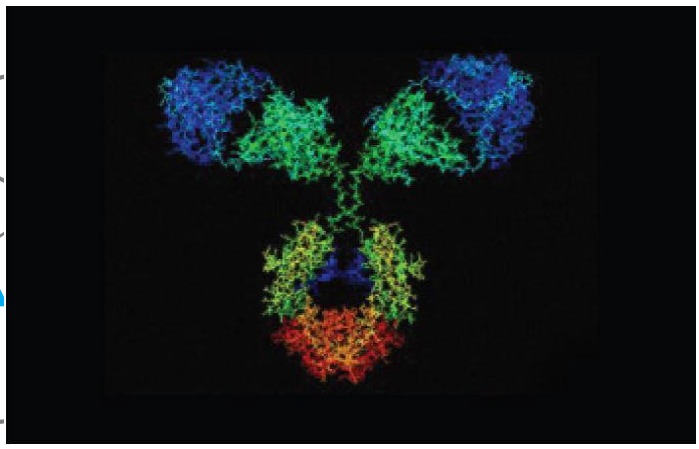
Ultra Fast Conformational XL-MS Epitope Mapping



CovalX offers a unique analytical service for epitope mapping based on its patented technology. Our service allows ultra-fast epitope characterization of monoclonal antibodies targeting any type of antigen (No limit in molecular weight). Based on mass spectrometry, our epitope mapping service is faster than any other methodology and allows epitope characterization with high resolution. Our technology is adapted to any type of epitope (linear or conformational) and the use of mass spectrometry combined with cross-linking gives unique information on the structure of the antibody-antigen interactions, including paratope identification. In addition, our methods allows additional insight such as stoichiometry of the interaction (mono- or bi-valency), aggregation and integrity of the antibody and antigen.

Initial Sample Screening

Before the high-resolution analysis of the epitope and paratope can begin, CovalX performs High-Mass MALDI analysis on the antibody, antigen and the intact antibody/antigen complex.

The goals of these analyses are to verify:
1) The integrity of both the antibody and the antigen
2) The possible aggregation of the antibody
3) The possible multimerization of the antigen
4) The stoechiometry of the antibody/antigen interaction (monovalency, bivalency).

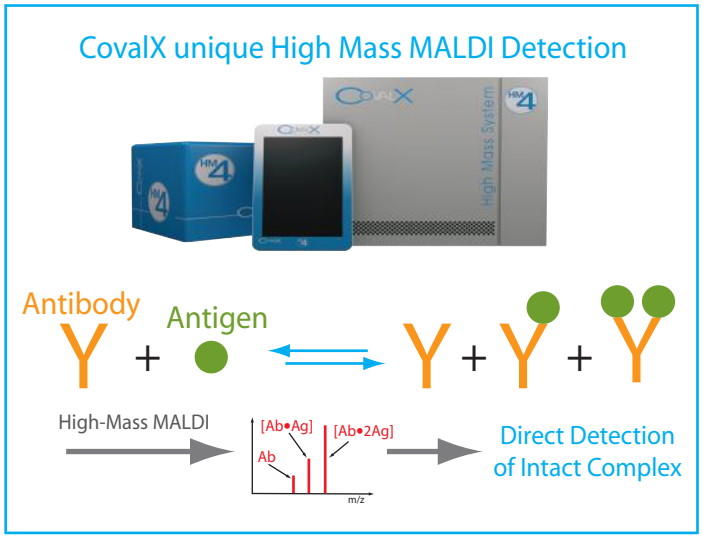


Figure 1. Direct detection of the unbound antibody and antigen as well as the intact complex is first determined using the CovalX unique High Mass MALDI detection systems.

Crosslinking Mass Spec Mapping (XL-MS)

After initial sample screening is completed, a selected cross-linker reagent, which is shown to effectively stabilize the complex (high mass MALDI analysis) is used for the epitope study. A 50:50 mixture of deuterated to undeuterated cross-linker is created to provide an opportune mass tag for detecting the linkers location within the sequences of both the antigen and the antibody.

Multiple Enzymes Provide Highest Coverage

After stabilization, multiple enzymes are utilized for comprehensive proteolysis. Trypsin, Chymotrypsin, ASP-N, Elastase and Thermolyse are each utilized individually to ensure the most thorough detection possible. The peptide coverage from the sum of these proteolysis provides the highest sequence coverage available with the most overlapping possibilities.

Finally, the peptide data is matched to their sequences using separate software packages to ensure proper identification of both the paratope and epitope from one data set.

•Nazabal et al. Anal. Chem., 2006, 78(11), 3562-3570
•Bich C et al. Anal. Biochem., 2008, 375:35-45

Why choose CovalX's Epitope Mapping Services?

- **Characterization of conformational or linear eptiopes**
- **Determination of both epitope and paratope**
- **Lowest cost commercial service available**
- **Only 150μg of each antigen/antibody required**
- **Four weeks delivery time**

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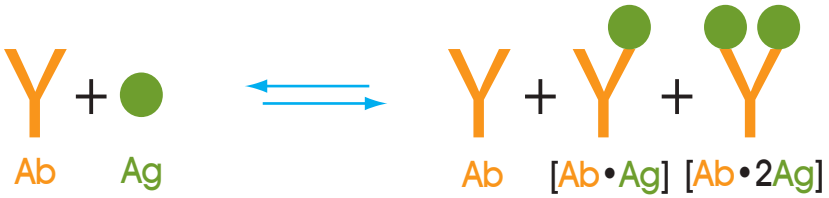
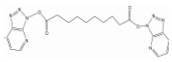
Epitope Mapping for Linear or Conformational Epitopes: 4 weeks



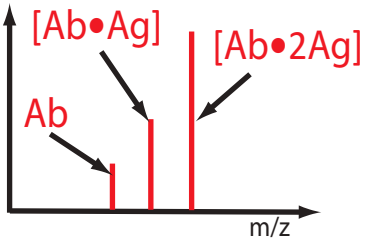
Antibody/Antigen Interractions

No limit in molecular weight for either the antibody or the antigen. The method is adapted to any protein-protein interaction.

Selected
Cross-linker
(50:50 deuterated)



High-Mass
MALDI MS
Analysis



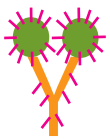
Direct detection of the
Immuno-complexes by High-Mass MALDI ToF

AB Sciex 5800 MALDI ToF ToF mass spctrometer
equipped with CovalX HM4 High-Mass system

High-Mass MALDI ToF MS

Using MALDI ToF MS equipped with High Mass system, it become possible to rapidly analyze non-covalent interactions in a very broad mass range (0-2000 kDa) with nM sensitivity

nLC-Orbitrap MS analysis of stabilized immuno-complexes



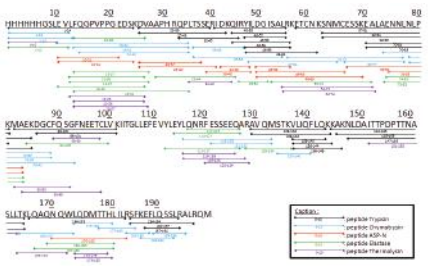
Purified Ab/Ag cross-linked complex

Multi-Enzymatic Proteolysis
(5 different enzymes)

Overlapping cross-linked
peptides

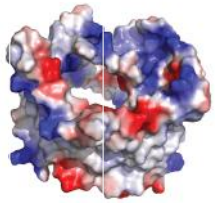
nLC-Orbitrap MS/MS analysis

Q-Exactive Orbitrap
High-Resolution
PMF



Data Analysis using
Interaction softwares
Detection of cross-link peptides
(Ab/Ag)

High-Resolution
Epitope map



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