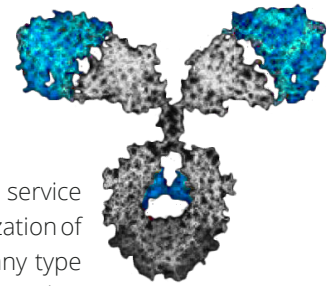
Ultra Fast Conformational XL-MS Epitope Mapping

Initial Sample Screening

Before the high-resolution epitope analysis begins, CovalX first performs High-Mass MALDI mass spectrometry analysis on the antibody (or similar), antigen and the intact protein complex. This initial screening utilizes CovalX's exclusive High-Mass MALDI detection systems to confirm that the XL-MS experiment is performed on well-characterized and controlled proteins and protein complexes.

The goals of these analyses are to verify:

1. The integrity of both the antibody and the antigen
2. Possible aggregation of the antibody
3. Possible multimerization of the antigen
4. The stoichiometry of the interacting proteins



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 interacting proteins.

Why choose CovalX's Epitope Mapping Services?

- Over a decade characterizing protein complexes by MS
- Experienced mass spec scientists overseeing all analysis
- Latest Automation & MS Instrumentation
- Reliable four to five weeks delivery time (RUSH options available)
- Proven professional results, used in patent filings

Crosslinking Mass Spec Mapping (XL-MS)

After initial sample screening is completed, a selected cross-linker reagent is used for the epitope study. This crosslinking reagent has been shown to effectively stabilize the protein complex.

Multiple Enzymes Provide Highest Coverage

After stabilization, multiple enzymes are utilized for comprehensive proteolysis. Trypsin, Chymotrypsin, Elastase and Thermolyse are each utilized individually to ensure the most thorough sequence coverage and 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 the epitope from the combined data set of all enzyme proteolysis.

*Nazabal et al. Anal. Chem., 2006, 78(11), 3562-3570

*Bich C et al. Anal. Biochem., 2008, 375:35-45

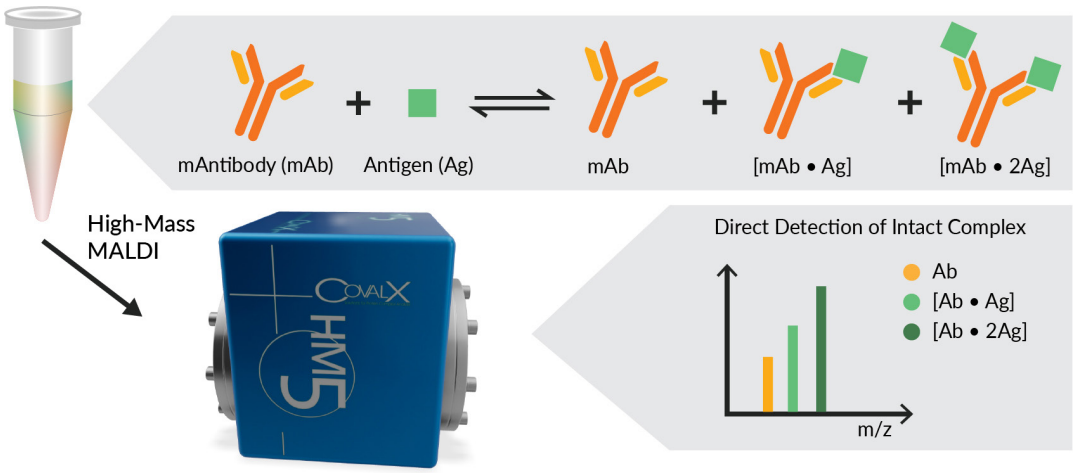


Figure 1
CovalX unique High-Mass MALDI Detection.

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

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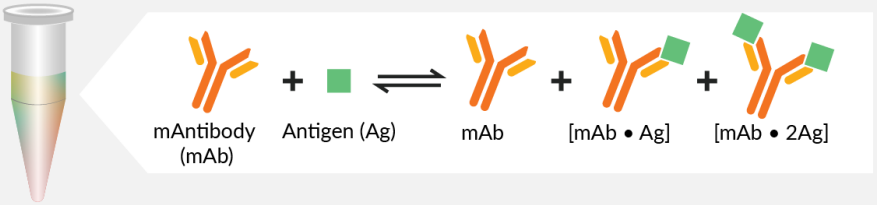
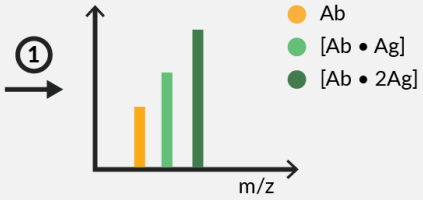
Epitope Mapping for Linear or Conformational Epitopes: 4 weeks



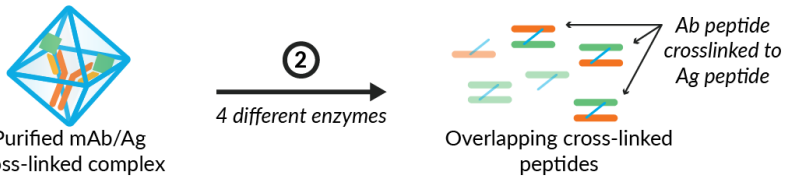


Antibody/Antigen Interactions. No limit in molecular weight for either the antibody of the antigen. The method is adapted to any protein-protein interaction.

Step 1: Selected Cross-linker (50:50 deuterated)

Direct detection of the Immuno-complexes using High-Mass MALDI ToF. Using MALDI ToF MS equipped with High-Mass system, it becomes possible to rapidly analyze non-covalent interactions in a very broad mass range (0-2000 kDa) with nM sensitivity.

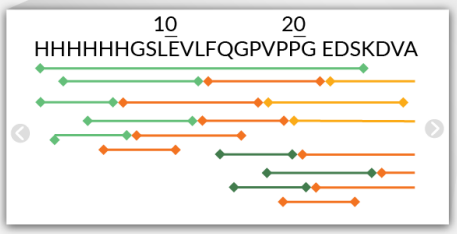
nLC-Orbitrap MS Analysis of Stabilized Immuno-complexes



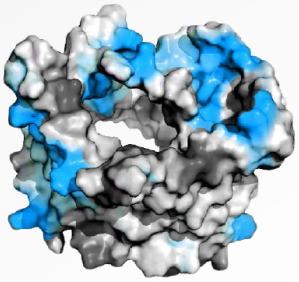
Step 2. Multi-Enymatic Proteolysis

Step 3. nLC-Orbitrap MS/MS analysis.

Step 4. Q-Exactive Orbitrap Characterization.



Step 6. Data analysis using interactions software. Detection of cross-linked peptides (mAb/Ag).



High Resolution Epitope Map

Switzerland | Schützengasse 23, Zürich CH-8001 | +41 44 585 3964
United States | 12 Gill Street, Suite 4850, Woburn, MA 01801 | +1 (617) 297 2263
France | 2, Allée Ulysse Gayon, 33650 Martillac | +33 7 66 86 59 50
www.covalx.com | info@covalx.com