

CovalX offers a unique analytical service for epitope mapping based on its patented technology. Our service allows ultra-fast epitope characterization of monoclonal antibody 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.

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 stoichiometry of the antibody/antigen interaction (monovalency, bivalency).

Rapid determination if Linear or Conformational

After the initial screening is passed, we determine the nature of the epitope: linear or conformational. If the recognition of the antigen is based on the tertiary structure of the antigen, the epitope is conformational. If the recognition is based on a linear sequence of the antigen, the epitope is linear.

To address the nature of the epitope, we are performing a fast competition assay using High-Mass MALDI mass spectrometry (Figure 1). First, the antigen is subjected to pepsin digestion in order to generate a large number of overlapping peptides covering the entire sequence of the antigen. Second, a competition assay is performed by mixing an excess of antigens pepsin digest, the intact antigen and the monoclonal antibody. After cross-linking and High-Mass MALDI analysis, the detection of the immunocomplex indicates the presence of a conformational epitope. If the immunocomplex is no longer detected, then the epitope to be characterized is linear.

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

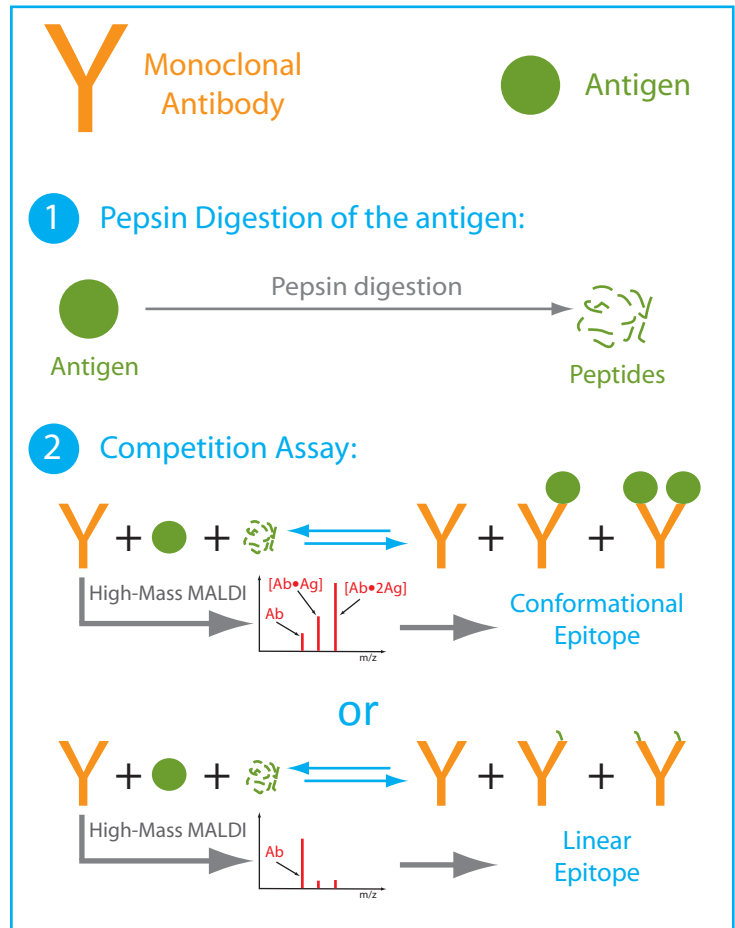
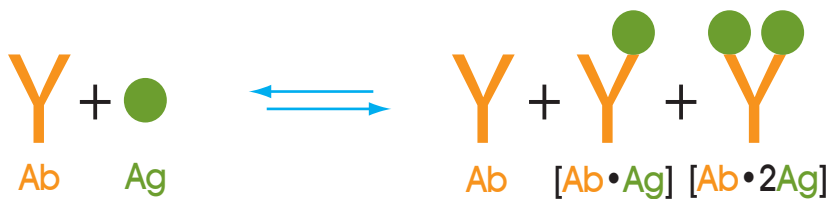


Figure 1. Characterization of the epitope (Linear or Conformational) by High-Mass MALDI ToF mass spectrometry.

Why choose CovalX's Epitope Mapping Services?

- Characterization of conformational or linear epitopes
- Determination of both epitope and paratope
- Lowest cost commercial service available
- Only 200µg of each antigen/antibody required
- Four to five weeks delivery time

Epitope Mapping for Linear or Conformational Epitopes: 4-5 weeks



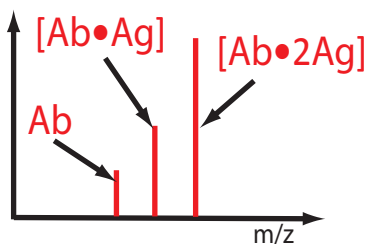
Antibody/Antigen Interactions

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 mass spectrometer equipped with CovalX HM4 High-Mass system

High-Mass MALDI ToF MS

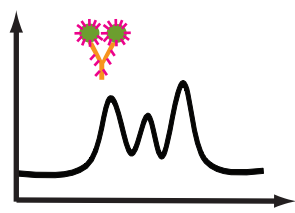
Using MALDI ToF MS equipped with High Mass system, it become possible to rapidly analyze non-covalent interactions in a very broad mass range (0-1500 kDa) with nM sensitivity

Purification of the Cross-Linked Complex



Antibody/Antigen cross-linked mixture

Size Exclusion Chromatography

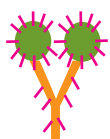


Size Exclusion Chromatography: Purification of the AB/Ag cross-linked complex

Size Exclusion Chromatography

In order to improve the detection of cross-link peptides, the intact Ab/Ag cross-linked complex is purified by SEC before any proteolysis.

nLC-Orbitrap MS analysis of stabilized immuno-complexes



Purified Ab/Ag cross-linked complex

Multi-Enzymatic Proteolysis (3-5 different enzymes)



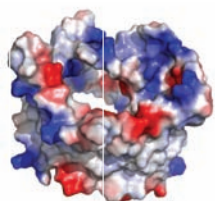
Overlapping cross-linked peptides

nLC-Orbitrap MS/MS analysis



High-Resolution PMF

High-Resolution Epitope map



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

