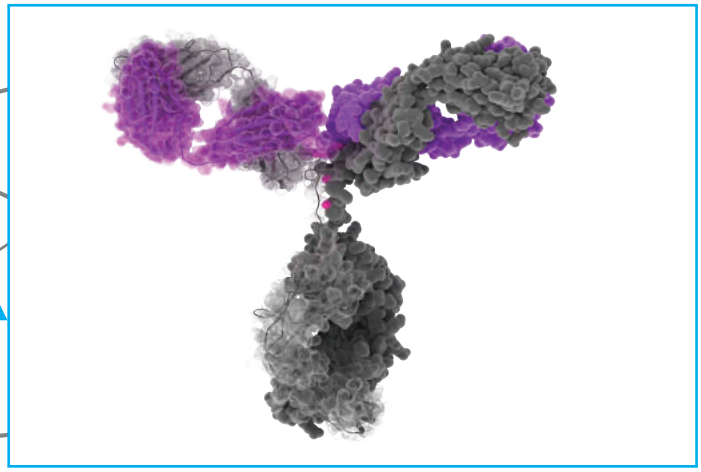
Hydrogen Deuterium eXchange (HDX) Mass Spec



Initial Screening of Protein and Complex

Before the high-resolution analysis of the epitope begins, CovalX first performs High-Mass MALDI analysis on the antibody, antigen and the intact antibody/antigen complex. This initial screening utilizes CovalX exclusive High-Mass MALDI detections systems to ensure that the HDX experiment is performed on characterized and controlled protein complexes.

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 protein complex

CovalX unique High Mass MALDI Detection

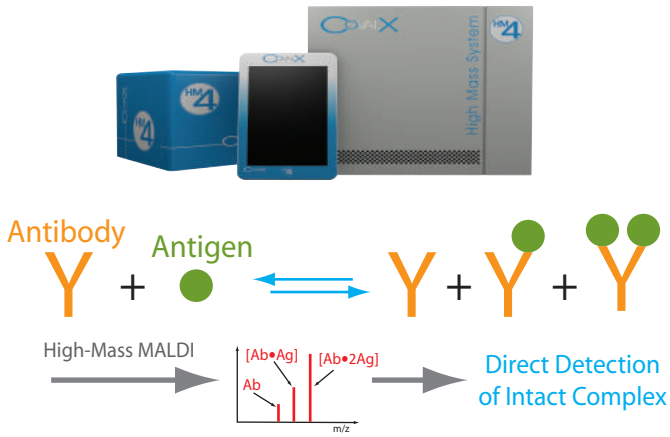


Figure 1. Direct detection of the unbound antibody and antigen as well as the intact complex is first determined using the CovalX unique High Mass MALDI detection systems.

CovalX offers unique analytical services for epitope mapping based on mass spectrometry. With over a decade specializing in mass spec characterization of protein complexes, CovalX offers unique expertise in the field of epitope mapping. Having developed unique technologies, our epitope mapping service provides reliable results on a scheduled timeframe. In addition, our technologies provide additional insight such as stoichiometry of the interaction (mono- or bi-valency), aggregation and antibody integrity.

Hydrogen Deuterium eXchange (HDX)

When diluted in heavy water (D_2O), backbone hydrogens of amino acids exchange with deuterium at varying kinetics rates depending upon their hydrogen bonding and solvent accessibility. When the antigen is complexed with the antibody, this deuterium uptake rate is altered in the epitope regions. These differences can be measured accurately at various time points.

1) After initial screening, the unbound antigen and the anibody:antigen complex are diluted in a D_2O solution.
2) At various time points the Deuterium/Hydrogen exchange is quenched at 0°C and pH 2.5.
3) The protein samples are then digested into peptides using appropriate proteolytic digestion.
4) The resulting peptides are then directly injected for microflow LC MS/MS detection.
5) From the peptide mass fingerprints (PMF), deuterium exchange rate heat maps can be compared between the antigen alone or the antigen with antibody binding.

These steps are all performed using the latest fully automated HDX processes. Sample handling is conducted using the LEAP H/D-X PAL™ automation. Efficient processing is conducted using its design of experiment (DOE) software. The entire analysis is conducted under temperature controlled conditions to further reduce hydrogen back-exchange of the deuterium during analysis. All analysis is overseen by experienced scientists with decades of background conducting HDX experiments. Finally, data is analyzed in HDExaminer software with full report generation using easy to understand HDX heat map descriptions and personal result presentation.

Why choose CovalX's Epitope Mapping Services?

- **Over a decade characterizing protein complexes by MS**
- **Experienced HDXMS scientists overseeing all analysis**
- **Latest Automation & MS Instrumentation (2016)**
- **Reliable six to eight weeks delivery time**
- **Proven professional results**

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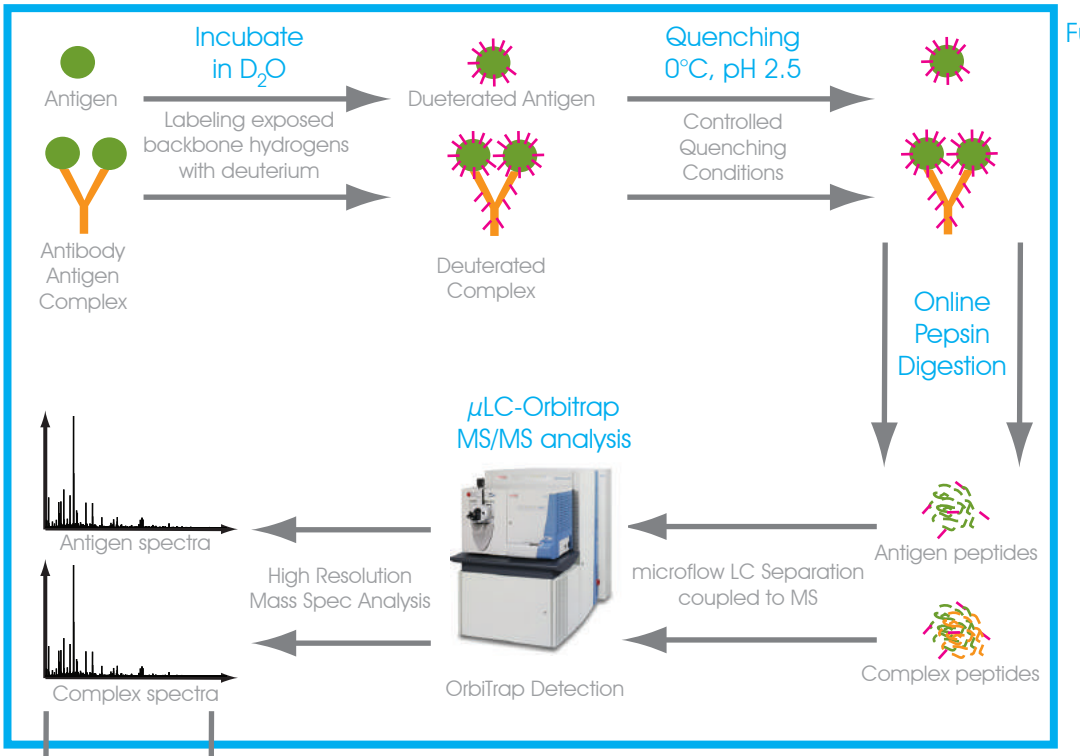
Conformational Epitope Mapping by HDXMS: 6-8 weeks

Initial Screening of Proteins and Intact Complex

High-Mass MALDI ToF MS

Using MALDI ToF MS equipped with CovalX a High Mass system to rapidly analyze non-covalent interactions directly.

Deuterium Labeling, Quenching, Digestion & Detection



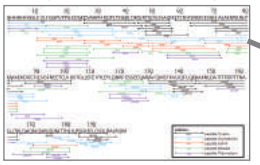
Fully Autonomous Process

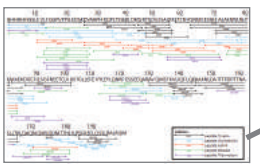
- Robotically controlled incubation, quenching & digestion conditions.

LEAP H/D-X PAL™ Automation

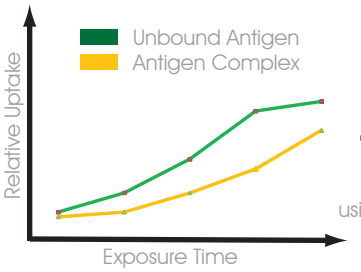
- Latest Design Of Experiment (DOE) software automation
- Rapid automation allows repeatability for time course experiments

Peptide Mass Fingerprints (PMF)


PMF of Antigen

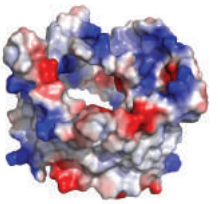

PMF of Complex

Comparison of Results



Data Analysis and Full Report Generation using HDExaminer software

High-Resolution Epitope Map



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