

# Hydrogen Deuterium eXchange (HDX) Mass Spectrometry

## CovalX Epitope Mapping services

CovalX offers unique analytical services for epitope mapping based on mass spectrometry. With over a decade of experience specializing in mass spectrometric 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.

Before the high-resolution analysis of the epitope begins, CovalX first performs High-Mass MALDI mass spectrometry analysis on the antibody, antigen and the intact antibody/antigen complex. This initial screening utilizes CovalX's exclusive High-Mass MALDI detection 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. Possible aggregation of the antibody
3. Possible multimerization of the antigen
4. The stoichiometry of the intact protein complex

## Hydrogen Deuterium eXchange (HDX)

When diluted in heavy water ( $D_2O$ ), backbone hydrogen from 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 due to the different solvent accessibility at the interaction site.

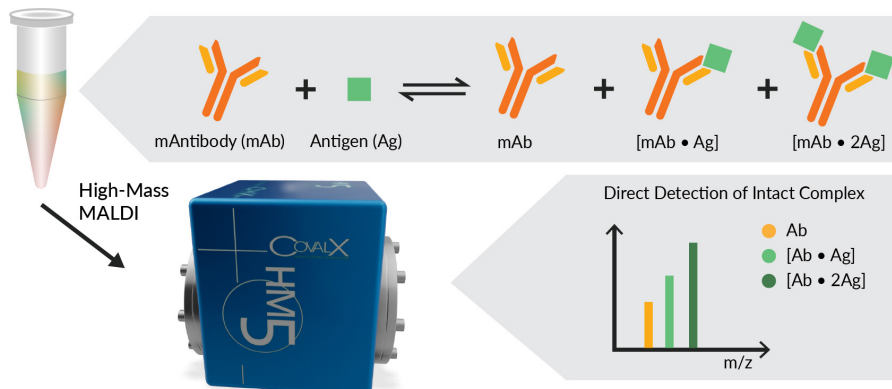
## Why choose CovalX's Epitope Mapping Services?

- 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

These differences will be measured accurately at various time points.

1. After initial screening, the unbound antigen and the antibody:antigen complex are each diluted in a  $D_2O$  solution.
2. At various time points the Deuterium/Hydrogen exchange is stopped by quenching the reaction (at  $0^\circ C$  and pH 2.5)
3. The protein samples are then digested in quenching conditions.
4. The resulting peptides are then directly injected for microflow LC and high resolution MS analysis.
5. From the peptide mass fingerprints (PMF), deuterium exchange rate heatmaps can be compared between the antigen alone versus the antigen complexed with the antibody.

These steps are all performed using the latest fully-automated HDX instrumentation. Sample handling is conducted using robotic 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 and ensure reproducibility. All analysis is overseen by experienced scientists with decades of background conducting HDX experiments. Finally, data is analyzed with dedicated software with full report generation using easy to understand HDX heatmap descriptions and personal result presentation.

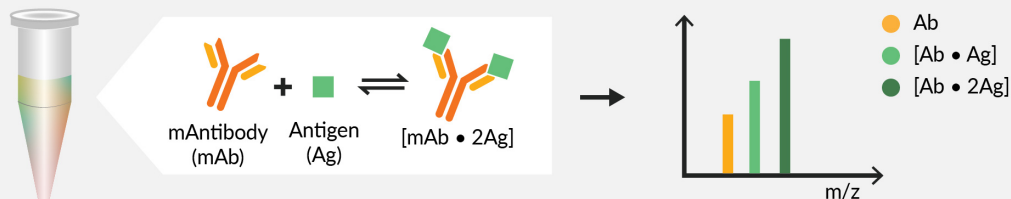


**Figure 1**  
CovalX unique High-Mass MALDI Detection.

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.

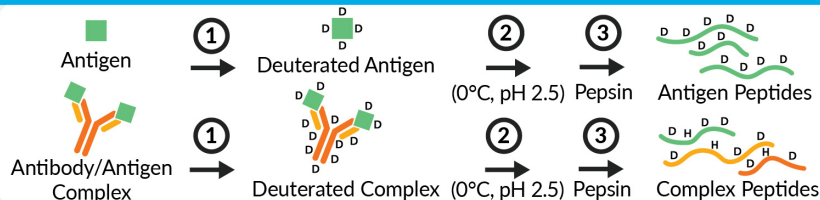
# Hydrogen Deuterium Exchange (HDX) Mass Spec

## Conformational Epitope Mapping by HDXMS: 6-8 weeks



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

## Deuterium Labeling, Quenching, Digestion & Detection

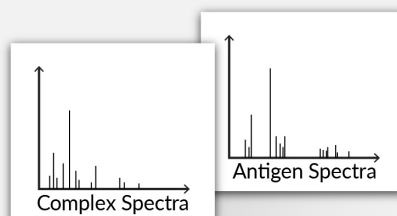


### Fully Autonomous Process

- Robotically controlled incubation, quenching, & digestion conditions.

### Latest HDX Automation Robotics

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



High Resolution Mass Spec Detection and Analysis

**μLC-MS/MS Analysis**  
Microflow LC Separation coupled to MS



## Data Analysis

### Peptide Mass Fingerprints (PMF)

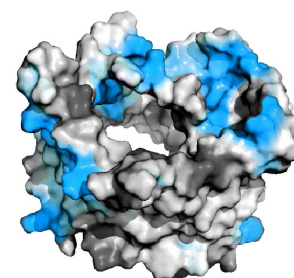
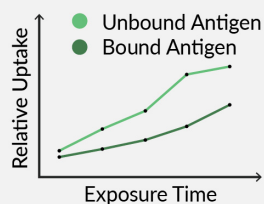


PMF Antigen when alone



PMF Antigen when mix with mAb

### Comparison of Results



Data Analysis and Full Report Generation using latest softwares for **High Resolution Epitope Map**