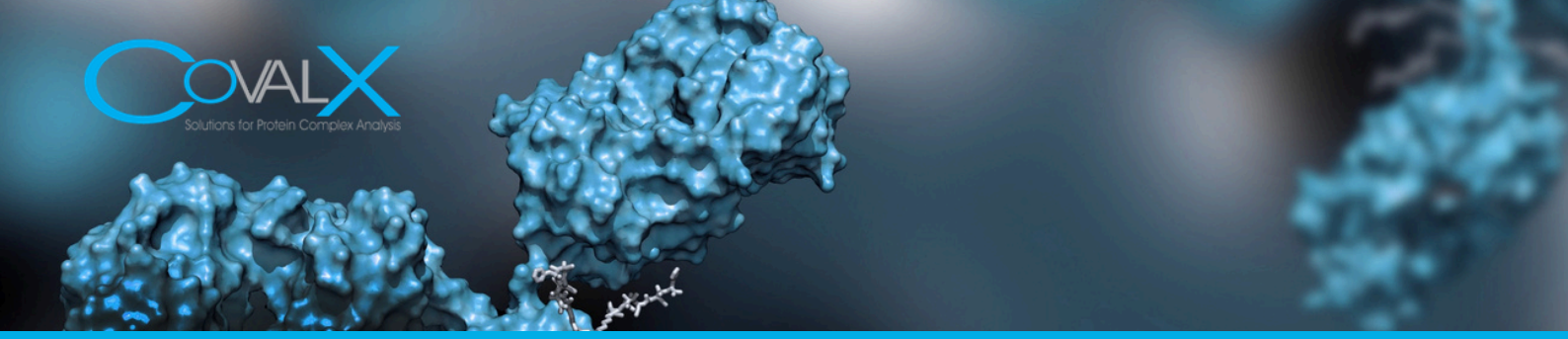


HDX-MS: Epitope Mapping

Epitope Mapping

Defining where a therapeutic antibody binds its antigen determines what the molecule does, and where it sits in a competitive landscape. HDX-MS maps binding sites by measuring solvent protection: the regions of the antigen that exchange hydrogen for deuterium more slowly in the presence of the antibody identify the contact interface. The method resolves linear and conformational epitopes in solution, under native conditions, and detects allosteric conformational changes induced by binding.

Hydrogen Deuterium Exchange (HDX)

When diluted in heavy water (D_2O), the backbone amide hydrogens exchange with deuterium at rates that depend on hydrogen bonding and solvent accessibility.

1. The unbound antigen and the antibody/antigen complex are each diluted in D_2O.
2. At each time point, the exchange is stopped by quenching the reaction (0 °C, pH 2.5).
3. The proteins are digested under quench conditions.
4. The resulting peptides are injected directly for microflow LC and high-resolution MS analysis.
5. Deuterium uptake per peptide is compared between the antigen alone and the antigen bound to the antibody, and displayed as heatmaps.

Sample handling for our HDX-MS experiments is conducted using the latest automation robotics, ensuring reproducibility across time points and conditions, and experienced HDX-MS scientists oversee every analysis. Data is processed with dedicated software, and the full report includes HDX-MS heatmaps and a presentation of the results to your team.

Why choose CovalX HDX-MS services?

- Results in 3 to 4 weeks from sample receipt.
- Systematic Hight-Mass MALDI QC.
- Allosteric changes captured alongside direct contacts
- Linear and conformational epitopes in a single experiment
- Automated labeling, quenching, and digestion for reproducible results.
- Every dataset reviewed by HDX-MS experts.

HM MALDI-MS: protein complex validation

HDX-MS data generated on an incomplete or heterogeneous sample is uninterpretable. 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 patented High-Mass MALDI detection systems to ensure that the HDX-MS 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.

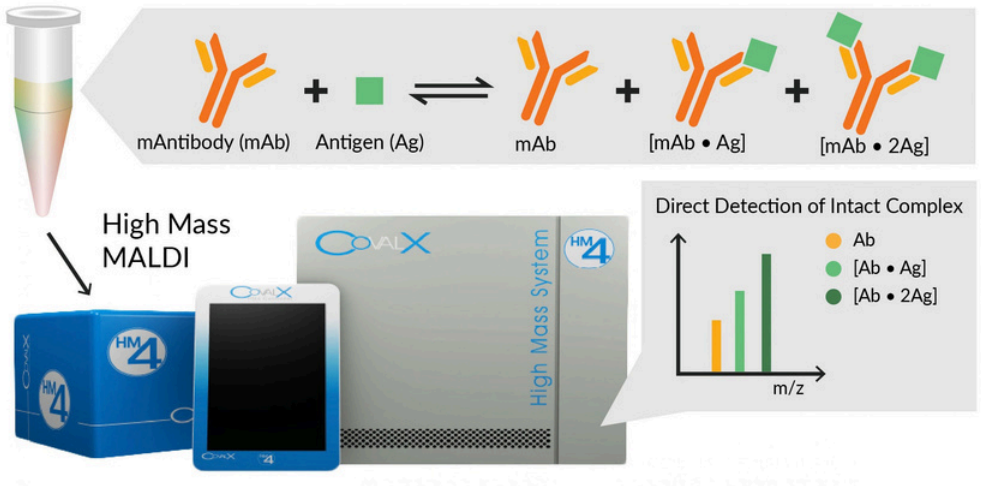
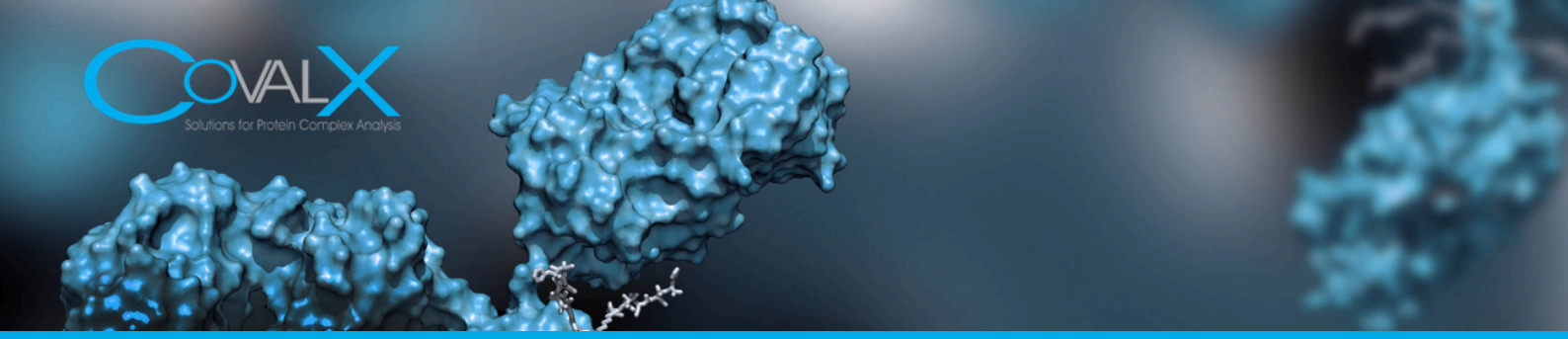
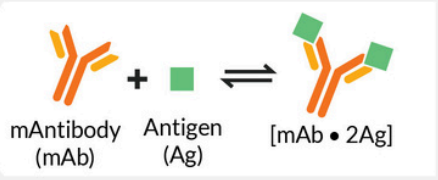


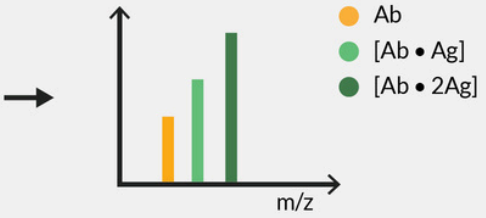
Figure 1
CovalX unique High-Mass MALDI QC step.
Direct detection of the unbound antibody and antigen as well as the intact complex is first determined using the CovalX unique High Mass MALDI detection systems.



Conformational Epitope Mapping by HDXMS: 6-8 weeks



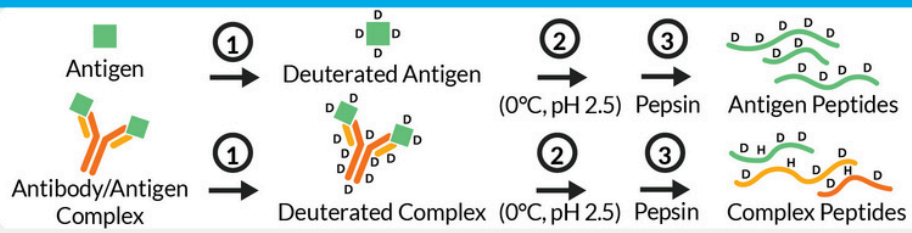




High-Mass MALDI ToF MS. Using MALDI ToF MS with CovalX, a High Mass system to rapidly analyze non-covalent interactions directly.

Deuterium Labeling, Quenching, Digestion & Detection





Step 1. Incubate in D₂O. Labeling exposed backbone hydrogens with deuterium.
Step 2. Controlled Quenching Conditions.
Step 3. Online Pepsin Digestion.

Fully Autonomous Process

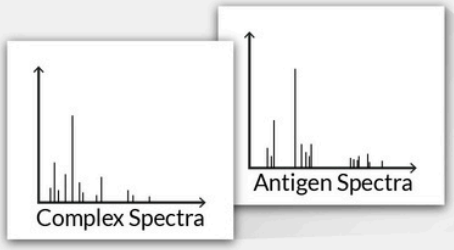
- Robotically controlled incubation, quenching, & digestion conditions.

Latest HDX Automation Robotics

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

µLC-MS/MS Analysis

Microflow LC Separation coupled to MS

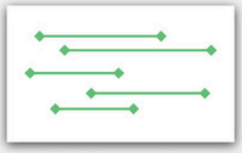


High Resolution Mass Spec Detection and Analysis

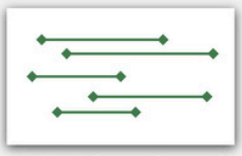


Data Analysis

Peptide Mass Fingerprints (PMF)

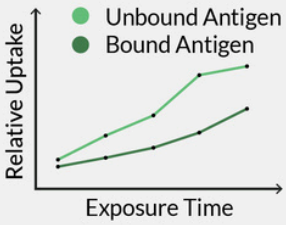


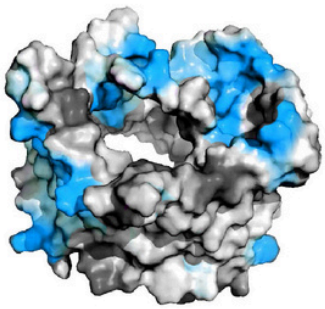
PMF Antigen when alone



PMF Antigen when mix with mAb

Comparison of Results





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

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