

Immunoglobulin protein [ca. 150,000 Daltons] participates in the immune reaction as the antibody for a specific antigen. There are five main types: IgG, IgM, IgA, IgE, and IgD.

Humanized IgG antibody fragment (Fab) [50,000 Daltons] VH, CH1 and CH2 regions, linked by an intramolecular disulfide bond.

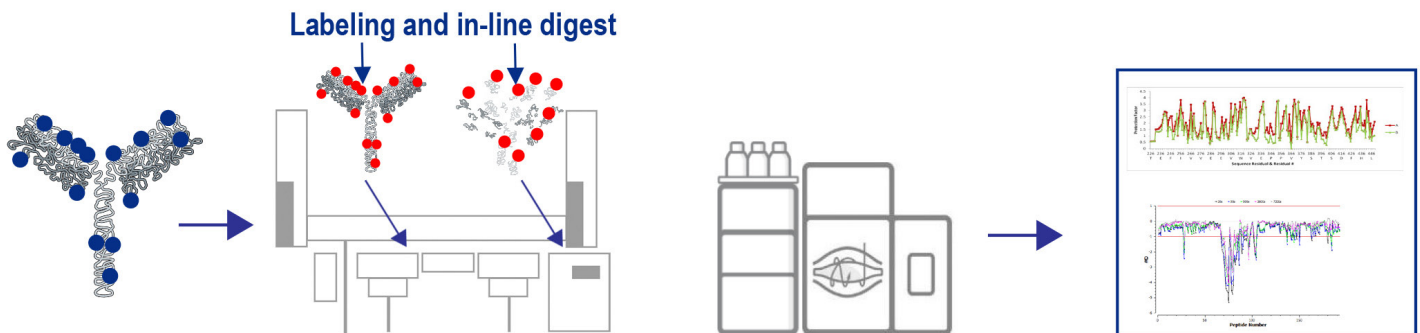
Higher Order Protein Structure Elucidation by HDX-MS

STRUCTURAL INSIGHTS

Sources of Charge Related Hydrophobicity	Effect	Species	Characterization	Result
Acidic	Protein aggregation	Acidic	Charge variation	Determined
Basic	Protein aggregation	Basic	Protein Aggregation	Determined
Adduct formation	COOH formation or loss of NH ₂	Acidic	Intact and Sub-unit Mass	Determined
Succinimide formation	Loss of COOH	Basic		
Methionine, cysteine, lysine, histidine, tryptophan oxidation	Conformational change	Basic		

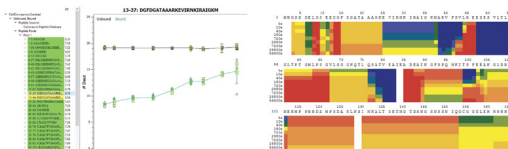
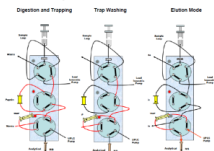
The Orbitrap Based Workflow Solution

The Thermo Scientific™ HDX Workflow comprises a HDx-3 PAL™ platform (Trajan Scientific and Medical) Hydrogen Deuterium Exchange sampler system and Thermo Scientific™ Vanquish binary pump N and binary pump F UHPLC system, coupled online with the Thermo Scientific™ Orbitrap™ family of mass spectrometers. The system is fully automated and controlled by Chronos Software (LEAP) fully integrated with Thermo Scientific™ Xcalibur™ software.



Any Orbitrap-based system can be coupled to the H/D-X PAL/LC front end

LEAP >>
TECHNOLOGIES



Sample handling

- Trajan Series II HDx Systems
- Chilled valve chamber for inline digestion, trapping and HPLC separation
- Secondary protease column chamber
- Flexible 3 valve configuration in the cooling chamber allowing efficient sample cleanup and column backflushing
- Capacity for filtration option
- Chronos software with full editing capabilities for method customization and full integration of Xcalibur software



LC Separation

- Vanquish Binary Pump N: all-in-one nano-, capillary, and micro-flow enables robust operation up to 1500bar
- Vanquish Binary Pump F: 1034 bar biocompatible high pressure gradient pump for sample loading
- Intelligent LC diagnostics and intuitive instrument method editor for simplified gradient programming



Mass spectrometer

- High resolution and accurate mass necessary for specificity with short chromatographic runs required to prevent back exchange and to allow precise measurement of deuterium incorporation
- High quality Orbitrap DDA spectra in conjunction with Thermo Scientific™ BioPharma Finder™ Software ensures confident and rapid identification of all peptides
- Ultimate precision with ETD or UVPD fragmentation to allow localization of deuterium exchange to the amino acid level
- The HDX-MS platform can be applied to run HDX and any other application



Data analysis

- BioPharma Finder: one software solution for peptide identification, PTM analysis and HDX data analysis.
- HDExaminer: a complete software solution for top-down and bottom-up HDX data analysis.
- Mass Spec Studio: AutoHX with DIA module introduces a complete solution for HX data analysis including high-quality peptide identification, analysis, manual curation and visualization

Learn more at thermofisher.com/HDX