

# Higher Order Protein Structure Elucidation by HDX-MS

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.

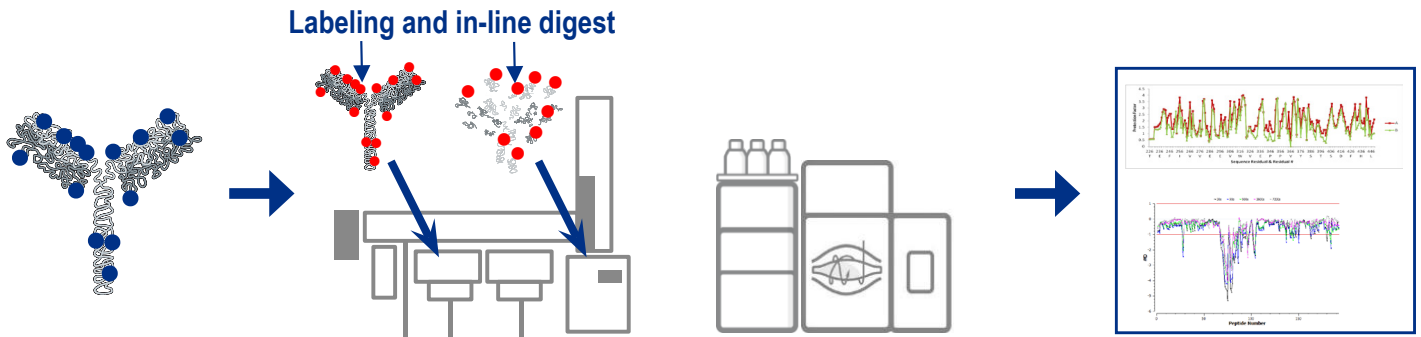
Humanized IgG antibody fragment (Fab) [ 50,000 Daltons ] VH, CH1 and CH2 regions, linked by an intramolecular disulfide bond.

STRUCTURAL INSIGHTS

Sources of Charge	Reduction	Effect	Species	Characterization	Result
Adduct formation	COOH formation or loss of NH <sub>2</sub>	Acidic	Charge Variance	Determined	
Succinimide formation	Loss of COOH	Basic	Protein Aggregation	Determined	
Methionine, cysteine, lysine, histidine, tryptophan oxidation	Conformational change	Basic	Intact and Sub-unit Mass	Determined	

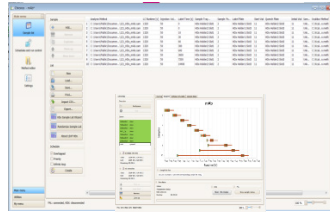
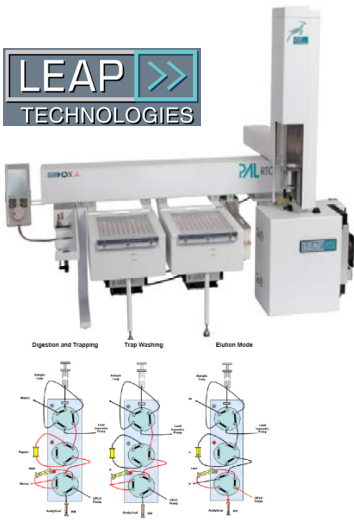
## The Orbitrap Based Workflow Solution

The Thermo Scientific™ HDX Workflow comprises a H/D-X PAL™ Hydrogen Deuterium Exchange sampler system (LEAP Technologies) and Thermo Scientific™ UltiMate™ 3000 UHPLC system, coupled online with the Thermo Scientific™ Orbitrap™ Platform of mass spectrometers. The system is fully automated and controlled by Chronos Software™ (LEAP Technologies) fully integrated with Thermo Scientific™ Xcalibur™ software.



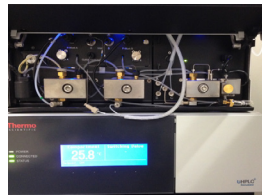
Any Orbitrap-based system can be coupled to the H/D-X PAL/LC front end





## Sample handling

- H/D-X PAL autosampler with single head with syringe exchange
- Standalone pepsin column chamber to enhance peptide coverage
- Closed chamber for stable temperatures for all vials
- Flexible 3 valve configuration in the cooling chamber allowing efficient sample cleanup
- Chronos software with full editing capabilities for method customization and full integration of Xcalibur software.



## LC pump

- Thermo Scientific™ Dionex™ UltiMate™ NCS-3500RS Binary Rapid Separation micro flow pump
- Ternary loading pump with flexible flow rate and solvent selection
- Fully editable LC conditions throughout the experiment

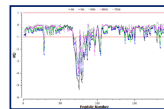
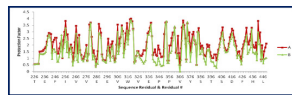
## Mass spectrometer

- 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 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.



Find out more at [thermofisher.com/HDX](http://thermofisher.com/HDX)

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