

# Structural Proteomics

## HDX-MS Principles

- Hydrogen-deuterium exchange
- Protein dynamics
- Conformational changes

## Cross-linking Constraints

- Distance measurements
- Protein topology
- Complex architecture

## Limited Proteolysis

- Protease accessibility
- Structural domains
- Folding states

## Ion Mobility

- Gas-phase separation
- Collision cross-section
- Shape information

## Detailed Techniques and Applications

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### Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS)

Principle

HDX-MS monitors the exchange of hydrogen atoms with deuterium in protein backbone amides. This exchange rate depends on hydrogen bonding, solvent accessibility, and protein dynamics.

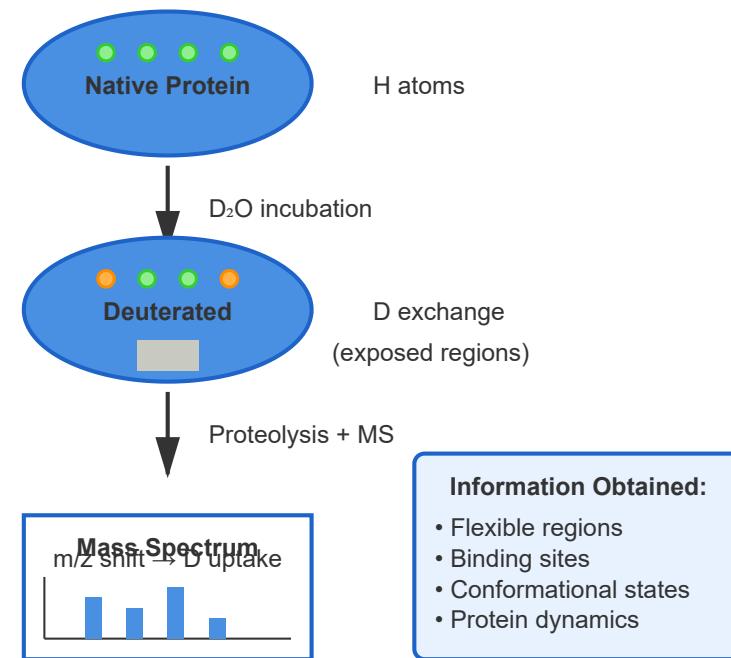
## Key Features

- **Exchange Kinetics:** Protected regions (secondary structures) exchange slowly, while exposed regions exchange rapidly
- **Time Resolution:** Milliseconds to hours, capturing multiple timescales of protein motion
- **Spatial Resolution:** Single amino acid level with optimized workflows

## Applications

- Protein-ligand binding interfaces
- Conformational changes upon activation
- Protein-protein interaction mapping
- Antibody epitope mapping
- Intrinsically disordered protein dynamics

## HDX-MS Workflow



## Principle

XL-MS uses chemical cross-linkers to covalently connect amino acids in close spatial proximity, typically within 10-30 Å depending on the cross-linker length.

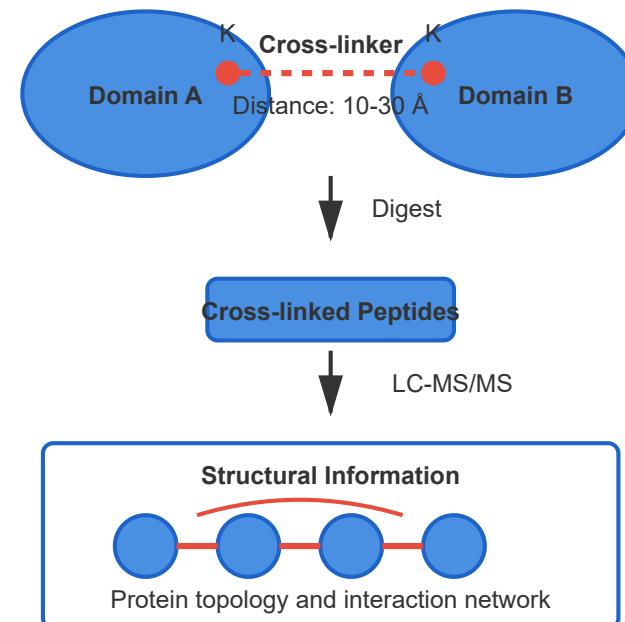
## Key Features

- **Distance Constraints:** Provides structural restraints for computational modeling
- **Cross-linker Types:** Lysine-lysine (DSS, BS3), zero-length (EDC), photo-reactive
- **Complex Analysis:** Captures transient and dynamic protein interactions

## Applications

- Large protein complex architecture
- Protein-protein interaction networks
- Membrane protein topology
- Intrinsically disordered regions
- In-cell structural studies
- Integrative structural biology

## Cross-linking Strategy



## 3 Limited Proteolysis Mass Spectrometry (LiP-MS)

# Principle

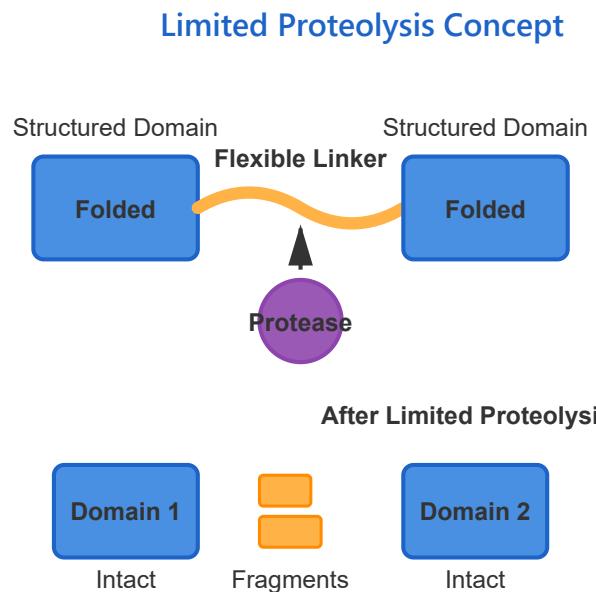
Limited proteolysis uses low concentrations of proteases under native conditions to selectively cleave exposed, flexible regions of proteins while leaving structured domains intact.

## Key Features

- **Structural Probing:** Differentiates folded from unfolded regions
  - **Native Conditions:** Maintains physiological protein states
  - **Protease Selection:** Commonly uses trypsin, proteinase K, or thermolysin
  - **Time-dependent:** Short incubation times preserve native structure

## Applications

- Domain boundary determination
  - Protein folding state assessment
  - Ligand-induced conformational changes
  - Protein stability analysis
  - Allosteric regulation studies
  - Quality control in biopharmaceuticals



## Mass Spectrometry Analysis

- ✓ Identify cleavage sites
  - ✓ Define domain boundaries
  - ✓ Map accessible regions
  - ✓ Assess protein stability

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# Ion Mobility Mass Spectrometry (IM-MS)

## Principle

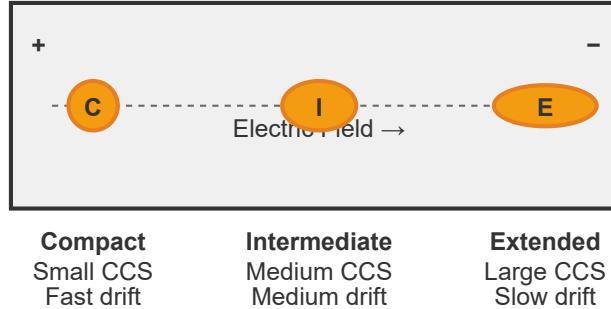
IM-MS separates ions based on their size, shape, and charge in the gas phase. Ions drift through an inert gas under an electric field, with compact structures traveling faster than extended conformations.

## Ion Mobility Separation

## Key Features

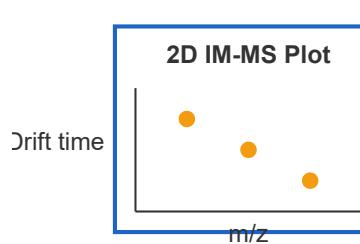
- Collision Cross-Section (CCS):** Measure of ion's surface area, related to 3D structure
- Conformer Resolution:** Separates different structural states of the same protein
- Gas-phase Analysis:** Rapid measurements (milliseconds)
- Native MS Compatible:** Preserves non-covalent interactions

## Ion Mobility Drift Cell



## Applications

- Protein complex stoichiometry determination
- Conformational ensemble characterization
- Protein folding pathway studies



## Obtained Data:

- CCS values
- Shape distribution
- Oligomeric states
- Conformational dynamics

- Aggregation and misfolding detection
- Structural validation for computational models
- Biopharmaceutical characterization