Protein Structural Analysis by Mass Spectrometry: Hydrogen Exchange and Covalent Labeling

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Obtain information about protein conformation and structure by selectively labeling proteins in solution, then using mass spectrometry to locate the label

Day 1: Protein structure refresher, as applied to MS

Methods of covalent labeling

Day 2: Methods of hydrogen exchange mass spectrometry
What can be learned from Hydrogen Exchange (HX) Mass Spectrometry (MS)?

- Protein folding pathways
- Proper folding of proteins
- Structural information
  - those that cannot be crystallized
  - those too big for NMR
  - those that are hard to prepare/purify
- Protein dynamics
  - where structures are mobile
  - where structures change
  - complexes, epitope mapping
  - conformational changes during function, binding, activation, etc.

Continuous Labeling HX MS Experiment

Equilibration
native conditions
(temp, pH, buffer)

Labeling reaction

Wait: multiple labeling times
(secs to hours)

Quenched reaction
0 °C, pH 2.6

Digest into peptides
pepsin, 0 °C, pH 2.6

Labeled protein(s)

Digest into peptides
pepsin, 0 °C, pH 2.6

Labeled peptides

Deuterium uptake

Mass spectra,
isotope pattern information

Interpretation

Chromatography
Reversed-phase, 8-40% ACN in 6 min.
0°C ice bath, pH 2.6 (to maintain label)
What can be learned from Footprinting MS?

- Protein folding
- Structural information
  - macromolecular assemblies that can not be crystallized or too big for NMR
  - membrane proteins
  - proteins in various physiological conditions
  - interactions of bulk, bound, ordered water
- Protein Dynamics
  - changes in protein structure
    > binding interfaces, complexes
    > conformational changes during activation, ligand binding, function, etc.
  - water dynamics within the transmembrane region
  - structure of mobile protein regions

Footprinting MS Experiment

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<tr>
<th>X-ray/Fenton</th>
<th>H₂O → •OH</th>
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Dose Response

MS/MS

LC-MS analysis

Fraction Unmodified

Abundance