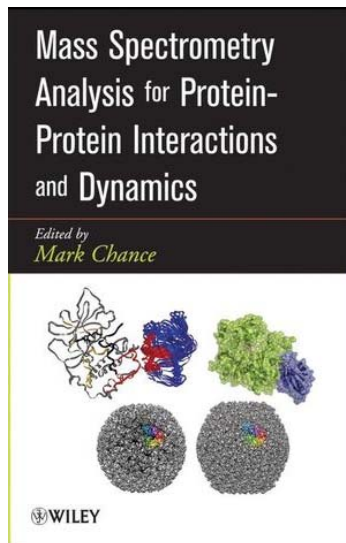


# Protein Structural Analysis by Mass Spectrometry: Hydrogen Exchange and Covalent Labeling

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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**



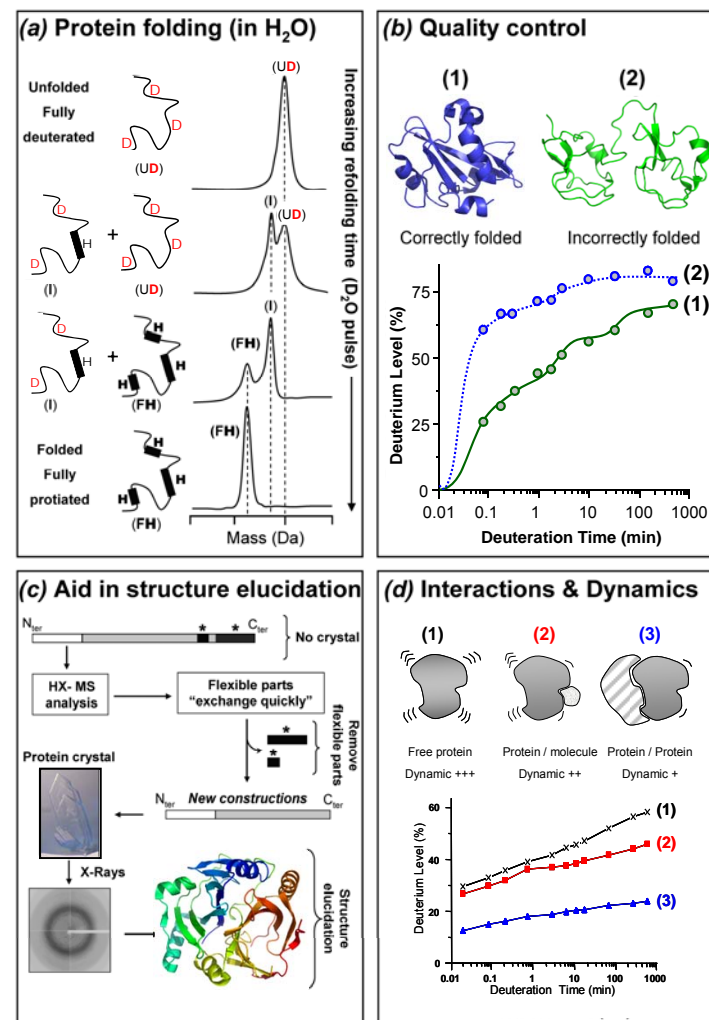
Course textbook

**Day 1: Protein structure refresher, as applied to MS  
Methods of hydrogen exchange mass spectrometry**

**Day 2: Methods of covalent labeling and crosslinking**

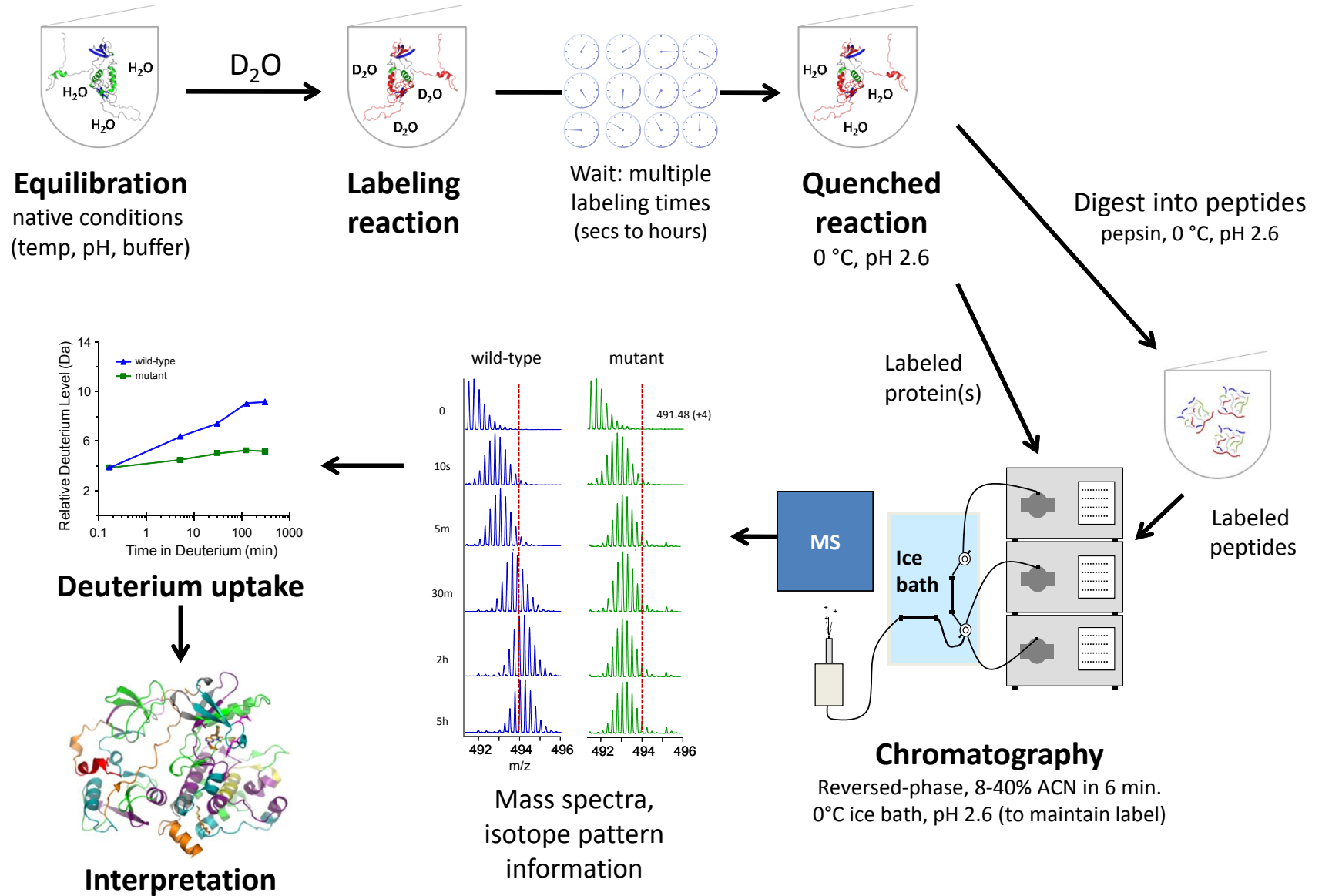
# What can be learned from Hydrogen Exchange (HX) Mass Spectrometry (MS)?

- 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.



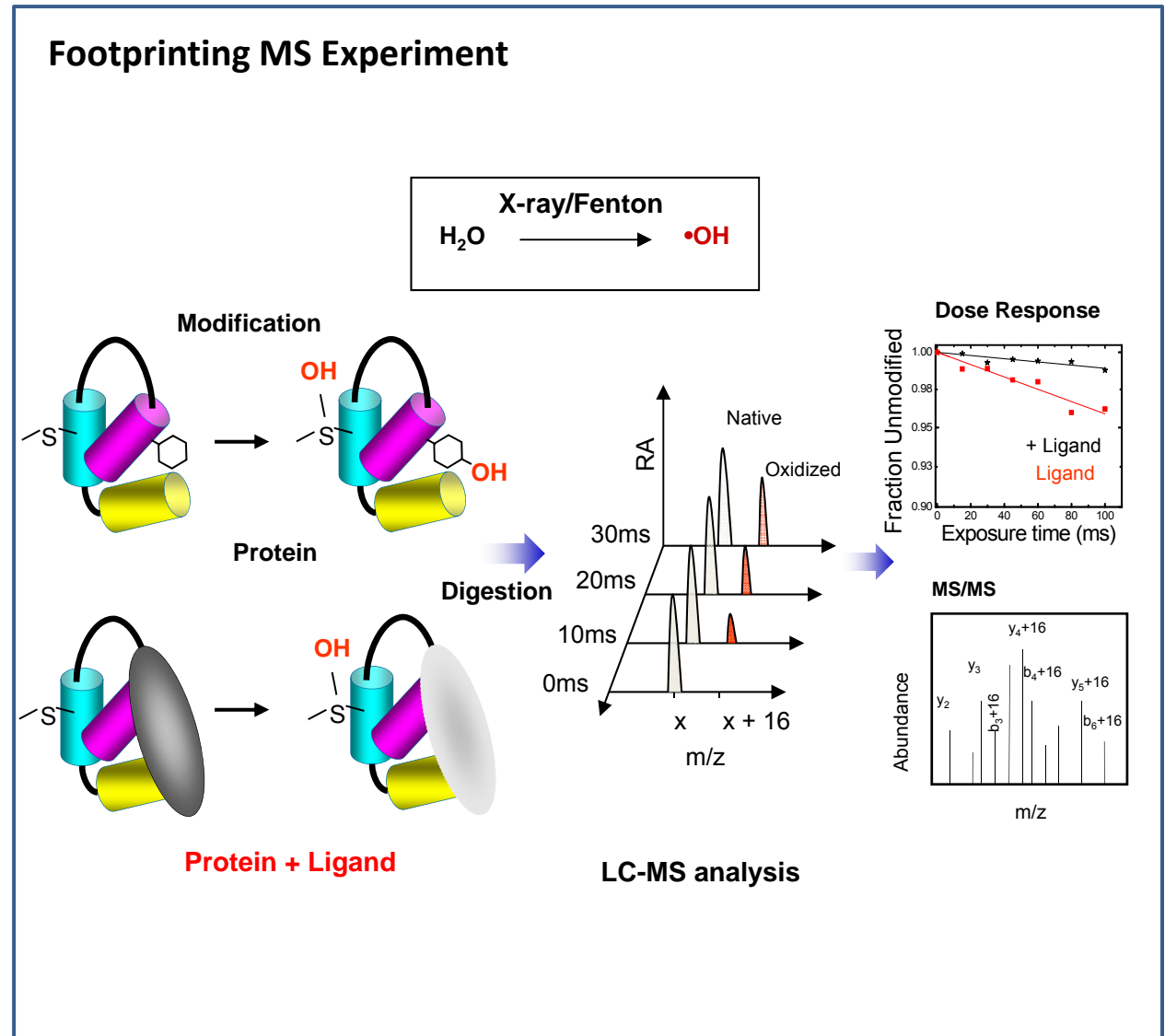
Hydrogen Exchange Mass Spectrometry: Principles and Capabilities. Brier S, Engen JR. In "Mass Spectrometry Analysis for Protein-Protein Interactions and Dynamics", 2008. Figure 2.6 p 33. ISBN: 978-0-470-25886-6, Blackwell Publishing, Mark R. Chance, Editor.

# Continuous Labeling HX MS Experiment



# 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



# What can be learned from Cross-linking MS?

- Structural information

- protein subunits
- conformational changes of proteins
- big protein assemblies that can not be crystallized or too big for NMR

- Protein interactions

- interactive analysis
- analysis of transient interactions
  - > in vitro cross-linking
  - > in vivo cross-linking

- Bioconjugation

- immunotoxin construction
- immunogenic preparation
- protein-protein conjugation

- Solid-Phase Immobilization

- DNA/RNA Cross-linking to proteins

