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56th ASMS Conference
June 2nd, 2008
Hydrogen Exchange: A Brief History

Linderstrøm-Lang (1950’s): “Amide hydrogen exchange rates should reflect the presence of hydrogen bonded structure”

Englander (1960’s): studied H/T exchange by liquid scintillation

(1980) improved the protocol by lowering the temperature of the separation step to reduce back-exchange.

Rosa & Richards (1979): Spacial resolution enhancement by combination of proteolysis with HPLC

Zhang and Smith (1993): combined HDX to MS, used FAB for ionization

Johnson and Walsh (1994): first to use ESI

Advantages over NMR & X-Ray

**X-Ray Crystallography**

- Crystallization problems
- Rigid systems

**Nuclear Magnetic Resonance**

- Low sensitivity
- High concentration required
- Protein size < 50 kDa

http://fig.cox.miami.edu/~cmallery/255/255tech/mcb3.38.xray.jpg

http://nmr.magnet.fsu.edu/facilities/900_105mm_TLH.htm
HDX at the molecular level

Low exchange rates:
α-helices, β-sheets and core of the protein.

High exchange rates:
Loops, turns and solvent accessible regions.
HDX at the macromolecular level

Types of HDX

A. Pulse Labeling

+ Perturbant

+ D₂O pulse

Fixed time (t)

pH 7.0, 25 °C

Equilibration (X hours)

Deuterium labeled protein

Quench: pH 2.5, 0 °C

B. Continuous Labeling

+ D₂O

10, 20, 30, 60 secs, etc

Multiple aliquot removal

Deuterium labeled protein

Quench: pH 2.5, 0 °C

Thomas E. Wales, John R. Engen, Mass Spectrometry Reviews, 2006, 25, 158–170
Types of HDX

C. Local Exchange Analysis

D. Global Exchange Analysis

Thomas E. Wales, John R. Engen, Mass Spectrometry Reviews, 2006, 25, 158–170
Types of HDX

• Off-exchange: it is hard to fully deuterate a protein, takes very long time…
• On exchange is preferred

Limitations of HDX

- Exchange in D$_2$O buffer while digestion, HPLC, MS in hydrogenated solvents and denaturing conditions.
- Use SFC$^1$, fast RPLC$^2$...
- Good sequence coverage
- Spatial resolution: multiple *overlapping* fragments.

Experimental Setup at NHMFL

- Protein or complex
  - Dilute 10 fold with D$_2$O buffer
- H/D exchange
- Quench pD 2.3 ~ 2.5
  - Digest with active enzyme at ~1.0 °C
- Peptide separation
  - ESI - MS
  - Time (min): 0.0, 0.5, 2.0, 60, 240, 4080
- Temp ~0 °C
- Desalt

Jasco HPLC/SFC System
The exchange rate constant

\[ k_{ex} = k_H[H^+] + k_{OH}[OH^-] \]

and \[ k_{OH} = 10^8 k_H \]

As temperature decreases, the exchange also decreases.

We need enzymes that work in these conditions!

PEPSIN – PROTEASES TYPE XIII & TYPE XVIII

David L. Smith, JOURNAL OF MASS SPECTROMETRY, VOL. 32, 1997, 135-146
Peptide Mapping

GSPEFGTGTGTRFGTDLAKEAKKVHQTTRTVPAKRGTIYDRNGVPIAEDATSYNV

YAVIDENYKSATGKILYVEKTQFNKVA...

Sequence coverage:

- **Pepsin**: 93%
- **Type XVIII**: 84%
- **Type XIII**: 40%

**99.7%**

Spatial resolution

Apomyoglobin Segment $F_{43}DKFKHLKTEAE_{54}$ from protease type XIII Cleavage

Applications

Folding and unfolding of proteins

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Influence of mutation on the 3D structures of proteins

Applications

Conformational change induced by ligand binding

Ligand: Ion, Metabolite or other Protein
