An Introduction to HDX Mass Spectrometry

Biophysical Lecture Series 2018
Glenn Mason

- Applications of the technique
- A little theory
- A example of a past project
- New advances in HDX-MS
Applications of the technique
I have this protein and...

You will need...
- Pure(ish) protein.
- About 300-500 μg of a 200 kDa protein – for the most basic questions as little as 100 μL of 5 μM protein.
- Buffer conditions without detergent or glycerol.
A little theory

Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS)

Measure the solvent exchange rate of backbone amide hydrogens in proteins.
Solvent exchange rate is dependent on a number of factors

Rate of exchange of amide protons is modified by many factors
1) pH (acid / base catalyzed)
2) Temperature
3) Primary sequence effects
4) Hydrogen bonds
5) Solvent accessibility
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HDX-MS measures the rate of exchange with solvent

Measure the solvent exchange rate of backbone amide hydrogens in proteins.

Timepoints between 0.3 s and 3000 s
Exchange reaction is quenched by lowering temperature and pH

Unfolded protein is digested

Use an acid-functional, non-specific protease in order to produce a diverse set of peptides in quenched conditions. Pepsin and Fungal XIII predominant choices (also nepenthesin).
Peptic peptide mass is measured

Separate Peptides on UPLC

Inject onto Mass Spec

Sample injection
Peptic Column
Peptide ‘TRAP’

Peptides stored in the trap.

15-30 minutes
Change Flow path

Reverse Phase UPLC

Peptides eluted (with the least hydrophobic first), and injected onto the Mass Spec.
Identification of deuterated peptides

\[ \text{wt PTEN} \\
35-42 \\
0 \text{ s HDX} \]

= Peak Centroid
Identification of deuterated peptides

Increasing incorporation of Deuterium with time

HDX-MS provides insight into dynamic structural changes
HDX-MS provides insight into dynamic structural changes

\[ \Delta m \] between the same peptide in different states is the useful information.
HDX-MS occupies a unique niche in bioscience

Advantages
- Theoretically unlimited size of protein*
- In solution measurement (rather than crystalline)
- Applicable for intrinsically disordered proteins
- Label free!

Disadvantages
- Resolution (dependent on the size of peptides)**

* Larger the protein, longer the data analysis
** Can be improved…

Available Instruments

Xevo
- "Old-Reliable"
- Manual injections
- External fluidics system, housed in ice.
- Possibly worse peptide identification, resolution and sensitivity…

Synapt
- Newer!
- Cool blue light!
- Allows both manual injections and automated sample handling.
- Internal fluidics system, chilled UPLC with a 16°C pepsin column.
- Possibly better peptide identification, resolution and sensitivity…
- Also has single-amino acid resolution HDX-MS capacity…
HDX-MS Examples

1) How protein complex interact with lipid membranes?

2) PI4K / Rab11 complex : HDX-MS before, during and after crystallography

3) How we can use HDX-MS/MS to gain insight into compound binding for PI3Kα
How protein complex interact with lipid membranes?

- Creating of Small Unilamellar Vesicles (SUVs) with biologically relevant lipid composition of phospholipids.
- How/where does my protein bind?
Experiment Design

Two datasets:
- Zero: Non-Deuterated Protein
- First: Protein Apo + D2O (without SUVs)
- Second: Protein with SUVs + D2

In total:
- Five timepoints (0.3s, 3s, 30s, 300s, 3000s) in triplicate
- Zero: 3 repeats
- First: 15 samples
- Second: 15 samples
  [Total = 33 samples]  Each sample = 30 mins

Total MS time = 15 hours instrument time
Total protein = 33 x 10 μl @ 5 μM = 330 μL @ 5 μM

Membrane binding example:
PTEN

1. PBM (4-21)  2. Arginine Loop (35-42)  3. WPD (82-99)  4. TI (155-177)
5. CBR3 (259-273)  6. CBR1 (201-215)  7. Cα2 (319-342)

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<th>Decrease</th>
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<tr>
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<tr>
<td>15-30%</td>
<td>&gt;30%</td>
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Membrane binding examples

PTEN (45 kDa)


PI3Kα (211 kDa)

Vps34 Complex II (340 kDa)

HDX-MS Examples

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Improving “crystallisability”

PI4KIIIβ: Rab11 crystallised unreproducibly, most likely due to proteasomal degradation.

Aims:
• Identify areas of intrinsic disorder to produce a crystal construct.
• Determine if PI4KIIIβ fold is maintained when intrinsic disorder is removed.
• Determine the interface between the two proteins.

Identifying Areas of Intrinsic Disorder in PI4KIIIβ

Step 1: Identify the sequence non-deuterated peptides of PI4KIIIβ (approx 2 h).
Step 2: Run 3 x 0.3 s deuterated samples of PI4K. (1.5 h)
Step 3: Determine areas of intrinsic disorder – peptides with a >50% incorporation of D₂O at 0.3 s (maybe half a day).
Is PI4K still folded properly?

![Graph]


What is the biologically relevant interface?

![Diagram]

A and B show possible binding sites.
Experiment Design

Four datasets:
- First and Second: to map the location of the Rab11 binding footprint on PI4KIIIβ (determined by having an excess (1.5x) of Rab11-GTPγS in solution) against PI4KIIIβ alone.
- Third and Fourth: to map the location of PI4KIIIβ binding footprint on Rab11 (determined by having an excess of PI4KIIIβ in the solution) against Rab11 alone.

In total:
- Four timepoints (0.3s, 3s, 30s, 300s) in triplicate for each experiment.
- Rab11 alone – 12 samples
- PI4K alone – 12 samples
- PI4K w/ 1.5x Rab11a – 12 samples
- Rab11a w/ 1.5x PI4KIIIβ – 12 samples
Total = 48 samples  Each sample = 26 mins
Total MS time = 21 hrs + approximately 5 hours for peptide ID

The interface
HDX-MS Examples

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Producing fragment ions with Collision Induced Dissociation

Precursor Ion  Collision Induced Dissociation  Fragment b & y ions

\[ \text{Precursor Ion} \rightarrow \text{Collision Induced Dissociation} \rightarrow \text{Fragment b & y ions} \]
HDX-MS stuck to peptide level resolution due to scrambling

Precursor ion

Collision Induced Dissociation

Randomised $b$ ions

Randomised $y$ ions

ETD vs CID

Precursor Ion

Collision Induced Dissociation

Fragment $b$ & $y$ ions

Precursor Ion

Electron Transfer Dissociation

Fragment $c$ & $z$ ions
ETD allows for ~0% scrambling fragmentation

First step: Standard HDX-MS with PI3Ka w/ & w/o compound (GDC-0941)
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- p110α
- p85α
- C-terminal Lobe
- N-terminal Lobe

- EECRIMSSAKRPLWL (-0.42 Da)
- FHIDFGHLDHPF (-0.35 Da)
- LIEVRSHTIMO (-0.71 Da)
Application of HDX-MS/MS

848-858 c ions

Peptide (IEVRSNTM)

Peptide FMKQMNDARHGGWTTKM

TOTAL ∆m = 2.5 Da

Residues F1039 to D1045 = 0.13 Da

Residues H1047 to M1055 = 2.1 Da

HDX-MS/MS provides insight into secondary structure

TOTAL ∆m = 2.5 Da

<0.1 Da<0.1 Da<0.1 Da

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0.5 Da0.5 Da0.5 Da

0.59 Da0.59 Da0.59 Da
You will need…

- Pure(ish) protein.
- About 300-500 μg of a 200 kDa protein – for the most basic questions as little as 100 μL of 5 μM (even 2.5 μM) protein.
- Buffer conditions without detergent or glycerol.
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More info at: glennmasson.com