An Introduction to HDX Mass Spectrometry

Biophysical Lecture Series 2019
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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.

Cys  Ser  Lys  Asp  Asn
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

![Amide exchange rate graph](image)

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![Hydrogen bonding and peptide chain](image)
Solvent exchange rate is dependent on a number of factors

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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

Quench at pH 2.5

0°C

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).

Digest Unfolded Protein

Pepsin Protease 3 min
Peptic peptide mass is measured

Sample injection

Pepsin Column

Peptide 'TRAP'

3 minutes

Peptides stored in the trap.

Change Flow path

Reverse Phase UPLC

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

Inject onto Mass Spec

Separate Peptides on UPLC

Xevo Synapt
Identification of deuterated peptides

Identification of deuterated peptides

wt PTEN
35-42
0 s HDX

= Peak Centroid

Relative Abundance

m/z

458 460 462 464 466 468
Identification of deuterated peptides

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)

Instrumentation
- Allows both manual injections and automated sample handling.
- Internal fluidics system, chilled UPLC with a 16°C pepsin column.
- Also has single-amino acid resolution HDX-MS capacity...
- Can make use of ion mobility spec also.
Instrumentation

- Ion Mobility Separation (IMS) cell allows for separation of ions via their size/shape also, allowing for another dimension of separation between ions
  - Cleaner spectra
  - Loss in signal
  - Can be useful for large (>200 kDa) samples in HDX

Data Analysis

- PLGS identifies peptides from non-deuterated Ms* (MS/MS data).

  - Typically use 3 samples to identify proteins.

  ~1 hr

  - Identify deuterated peptides
  - Calculate deuterium exchange rates

  Depends on the size of your protein, but at least a couple of days of manual (and boring) data processing
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 10μL @ 5μM = 330μL @ 5μM

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**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. Ca2 (319-342)

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<th>Decrease</th>
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<td>15-30%</td>
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<tr>
<td>5% to 5%</td>
<td>&gt;30%</td>
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Membrane binding examples

**PTEN (45 kDa)**


**PI3Kα (211 kDa)**
Burke JE, Perisic O, Masson GR, Vadas O, Williams RL. Oncogenic mutations mimic and enhance dynamic events in the natural activation of phosphoinositide 3-kinase p110α (PIK3CA). PNAS. doi: 10.1073/pnas.1205508109

**Vps34 Complex II (340 kDa)**

HDX-MS Examples

1) How protein complex interact with lipid membrane?

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

3) How we can use HDX-MS to monitor changes in protein function, binding for PI3Kα
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?

What is the biologically relevant interface?

Experiment Design

Four datasets:
- First and Second: to map the location of the Rab11a 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 Rab11a (determined by having an excess of PI4KIIIβ in the solution) against Rab11a alone.

In total:
- Four timepoints (0.3s, 3s, 30s, 300s) in triplicate for each experiment.
  - Rab11a 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

1) How protein complex interact with lipid membrane?
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

Producing fragment ions with Collision Induced Dissociation

Precursor Ion → Collision Induced Dissociation → Fragment b & y ions

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<th>b ions</th>
<th>y ions</th>
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Diagram showing the process of producing fragment ions with Collision Induced Dissociation.
HDX-MS stuck to peptide level resolution due to scrambling

**Precursor Ion**

Collision Induced Dissociation

Scrambling

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

Precursor ion Mass normally measured in HDX-MS

Electron Transfer Dissociation (ETD)

No Scrambling

First step: Standard HDX-MS with PI3Ka w/ & w/o compound (GDC-0941)
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- GDC-0941
- p110\textsubscript{α}
- p85\textsubscript{α}

C-terminal Lobe
N-terminal Lobe

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

HDX-MS/MS provides insight into secondary structure

TOTAL $\Delta m = 2.5$ Da

Residues F1039 to D1045 = 0.13 Da

Residues H1047 to M1055 = 2.1 Da
How big is big?

Where do GCN2 and the ribosome interact?
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HDX-MS on a Ribosome
Where does GCN2 Bind?
79 proteins
1.5 mDa of protein
>13,000 amino acids

Where do GCN2 and the ribosome interact?

3 Peptides Identified from HDX
All peptides found in the P-stlak
Where do GCN2 and the ribosome interact?

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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Acknowledgements

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More info at: glennmasson.com