Mar 19, 2025

Hydrogen-Deuterium Exchange Coupled to Mass Spectrometry of Clusterin

DOI
https://dx.doi.org/10.17504/protocols.io.e6nvwbxy7vmk/v1

Alonso I. Carvajal[1], George-Valentin Datcu[1], Andreas Bracher[1], Patricia Yuste-Checa[1], F. Ulrich Hartl[1]

[1]Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany

Patricia Yuste-Checa
MPI Biochemistry

DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwbxy7vmk/v1

Protocol Citation: Alonso I. Carvajal, George-Valentin Datcu, Andreas Bracher, Patricia Yuste-Checa, F. Ulrich Hartl 2025. Hydrogen-Deuterium Exchange Coupled to Mass Spectrometry of Clusterin. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwbxy7vmk/v1

Protocol status: Working
We use this protocol and it's working

Created: March 11, 2025

Last Modified: March 19, 2025

Protocol Integer ID: 124314

Keywords: ASAPCRN, mass spectrometry of clusterin, mass spectrometry, clusterin, deuterium exchange, hydrogen, hdx

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000282

Abstract

This protocol details how to perform hydrogen-deuterium exchange coupled to mass spectrometry (HDX-MS) of clusterin.

Materials

Buffers:

1. HDX buffer:

	A	B
	Na-acetate pH 5.0	20 mM
	NaCl	100 mM
	EDTA	1 mM
	Tris(2-carboxyethyl)phosphine (TCEP)	1 mM

2. Deuteration buffer: A volume of HDX buffer was subjected to two cycles of lyophilization/resuspension in an equal volume of D_2O.

Note

NB: Adjust pD to 5.0 (With a H_2O-calibrated pH-meter, the conversion of pH into pD is then accomplished by adding a constant of 0.4).

3. Quenching buffer: Keep on ice.

	A	B
	Sodium phosphate pH 2.4	100 mM
	TCEP	20 mM
	Guanidine-HCl	7 M

4. Sodium phosphate pH 2.4.
5. LC solvent A: 0.2% (v/v) formic acid
6. LC solvent B:

	A	B
	Acetonitrile	99% (v/v)
	Formic acid	0.1% (v/v)

7. LC wash solution: guanidine-HCl, 4% (v/v) acetonitrile

	A	B
	Guanidine-HCl	1.5 M
	Acetonitrile	4 % (v/v)
	Formic acid	1% (v/v)

Proteins:

WT-Clu or Clu-Δ(214–238) at 200 µM in HDX buffer

Exchange reaction

15m

1 Incubate proteins and deuteration buffer at 25 °C before starting the exchange reaction.

2 Dilute 2.2 µL of protein with 27.8 µL of deuteration buffer (92.6% D_2O) in an Eppendorf low binding tube.

3 Run the deuteration reactions for 10, 100, and 1000 s at 25 °C.

4 Stop the reactions by the addition of 79 µL of ice-chilled Quenching buffer.

5 Incubate the samples On ice for 00:15:00.

15m

6 Add 109.1 µL of sodium phosphate pH 2.4. This should result in a final pH between pH 2.5 and pH 2.6.

Note

Note: The quenching conditions were optimized for complete denaturation of clusterin and
maximum sequence coverage.

Sample injection

7

Note

Note: The program MassLynx suite 4.1 was used to operate the HDX-MS equipment, including the
HDX Manager, LC and MS.

Set the temperature of the chamber to 0 °C and the column temperature to 20 °C in the HDX Manager.

8 Wipe off the syringe needle with lint-free paper.

9 Rinse the syringe with a 50% methanol solution.

10 Rinse the syringe with LC solvent A three times.

11 Inject 200 µL of LC wash solution into the 50 µL loop via the LC inject port of the LC HDX Manager of the Synapt G2Si.

12 Run twice the saw-tooth wash methods.

13 Inject the full volume of the deuteration reaction into the 50 µL loop via the LC inject port of the LC HDX Manager of the Synapt G2Si.

Note

A HDMSE method was used in ion mobility mode.

14 Repeat steps 10-13 to measure all your samples.

Conditions used in the LC experiment

23m

15 Perform digestion using an Enzymate BEH-pepsin column (Waters) at a flow rate of 100 µL and temperature of 20 °C.

16 Trap and desalt peptides for 00:03:00 at 100 µL using an ACQUITY UPLC Peptide CSH C18 VanGuard Pre-column before transfer to an analytical 1.0 x 100 mm

3m

ACQUITY UPLC peptide CSH C18 column (Waters) held at 0 °C .

17 Elute peptides for 00:20:00 with an 8-40% acetonitrile gradient in 0.1% formic acid, pH 2.5 . 20m

Note

- Gradient was optimized to obtain a higher protein coverage.
- The analytical column was washed using two repeating saw-tooth gradients and equilibrated at 8% acetonitrile between injections to reduce carry-over.
- Mass analysis was performed on a Waters Synapt G2Si. T-wave ion mobility was used as an orthogonal peptide separation step between the UPLC and mass spectrometer. Ion guide settings were adjusted to minimize gas-phase back exchange.

Data analysis

18 Import raw files of undeuterated samples produced by MassLynx into Proteinlynx Global Server 3.0 (PLGS) for peptide search. Use a Fasta file containing the sequences of the protein tested and pepsin.

19 Import final_peptide.csv output file from PLGS into DynamX 3.0 along with the raw data of undeuterated samples for spectra identification and deuterium uptake calculation for all the samples.
Apply the following filters:

- minimum intensity of 1000 counts;
- maximum length of 25 amino acids;
- minimum length of 3 amino acids;
- minimum number of products per amino acid of 0.1;
- minimal score 6;
- maximum mass error of 10 ppm.

20 Import the rest of spectra of deuterated samples into DynamX.

21 Analyze each peptide spectrum individually and peak-wise manually in order to check their assignment.

22 Export the results as Pymol script to color PDB structure based on the HDX exchange observed.