Characterization Of Antibody-Drug Conjugates Using HDX and Limited Proteolysis-Mass Spectrometry

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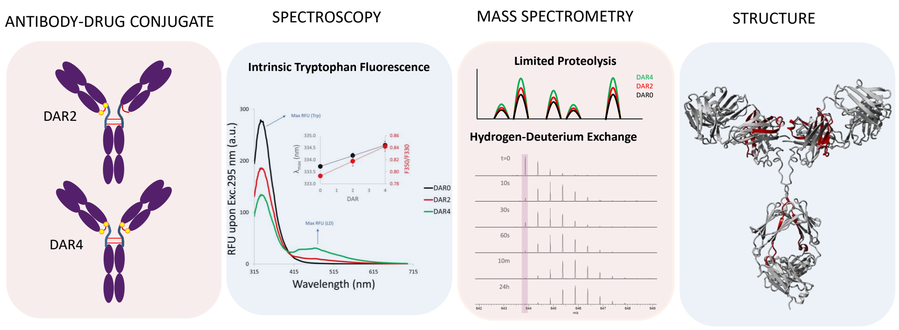
BACKGROUND

The efficacy of biopharmaceuticals, such as monoclonal antibody-drug conjugates (ADC), is strongly dependent on maintaining structural integrity throughout industrial process manufacturing, and this is traditionally monitored using **spectroscopic techniques**. Such methods monitor important aspects of protein structure but offer limited resolution regarding the exact sites of structural change. At the same time, **mass spectrometry (MS)-based methods** are gaining ground in the pharmaceutical industry because they allow peptides indicative of structural integrity to be detected with shotgun or bottom-up proteomics workflows in a single chromatographic run, and thus localize structural changes with increased accuracy. In this work, we set out to evaluate the complementarity of **limited proteolysis-MS** (LiP) and **hydrogen-deuterium exchange-MS** (HDX) for the analysis of protein **higher-order structure** (HOS) in a biopharmaceutical setup.

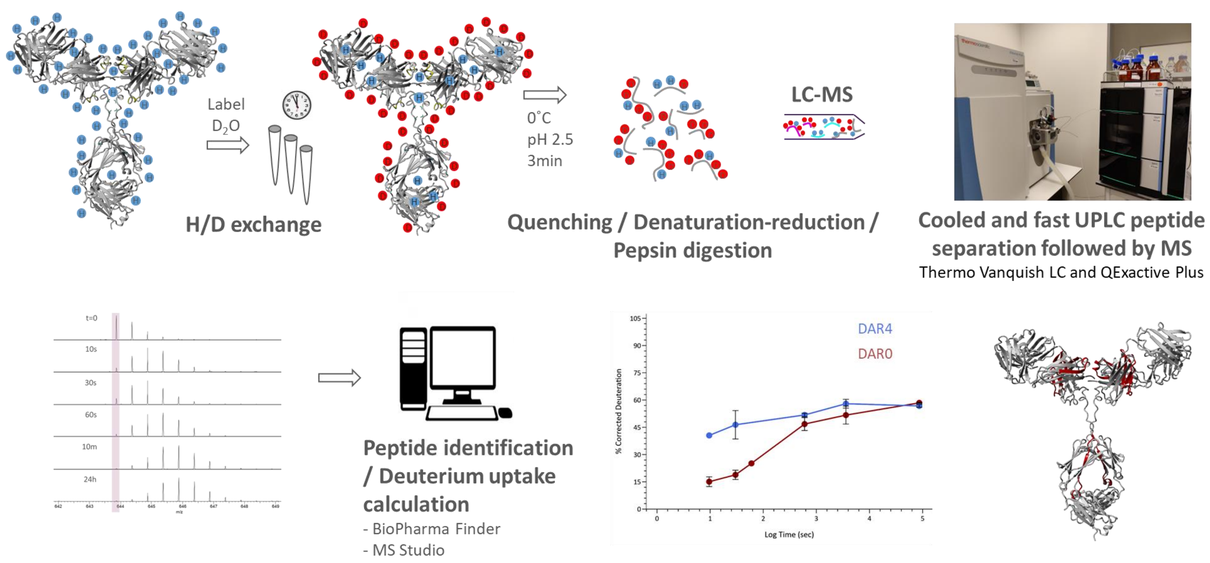
STUDY DESIGN



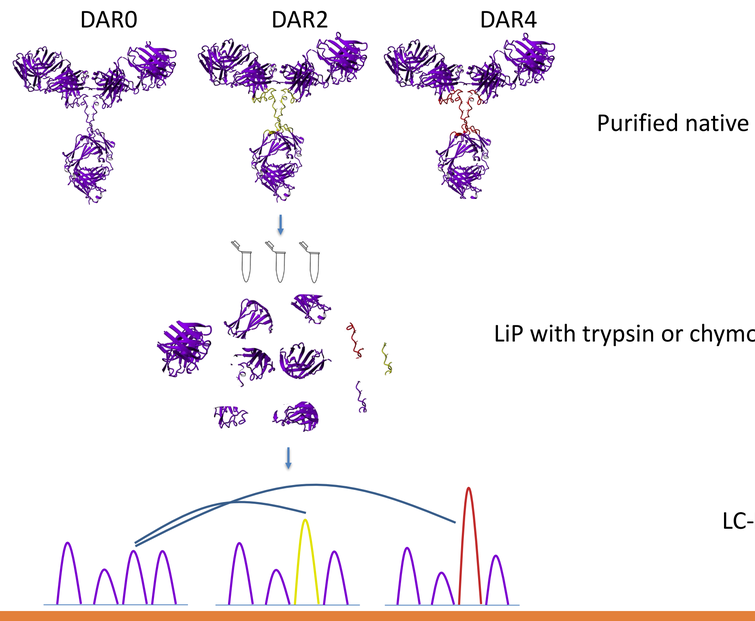
- Randomly-conjugated ADC species with different drug-to-antibody ratio were compared using Intrinsic Tryptophan Fluorescence spectroscopy
- LiP-MS was performed using two different proteolytic enzymes
- To increase resolution of conformational changes HDX-MS was used

METHODS

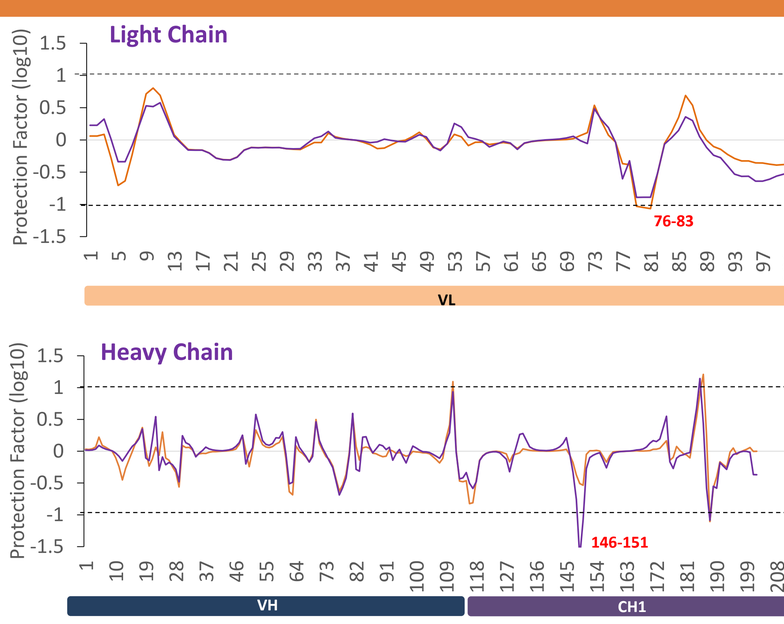
HDX-MS workflow



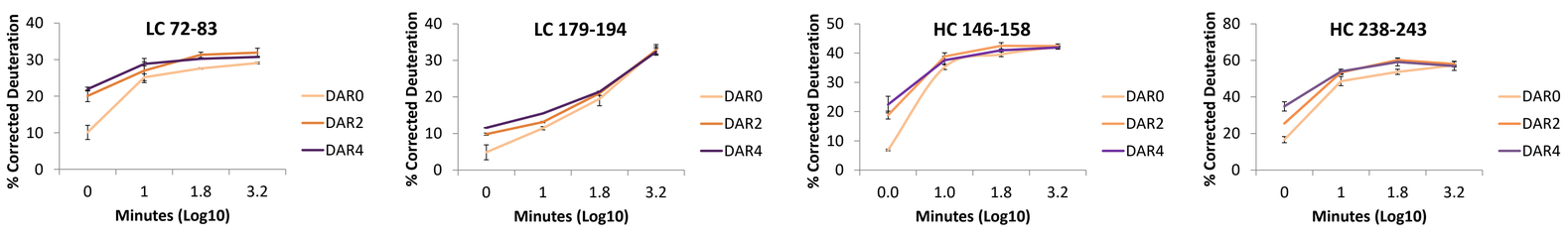
LiP-MS workflow

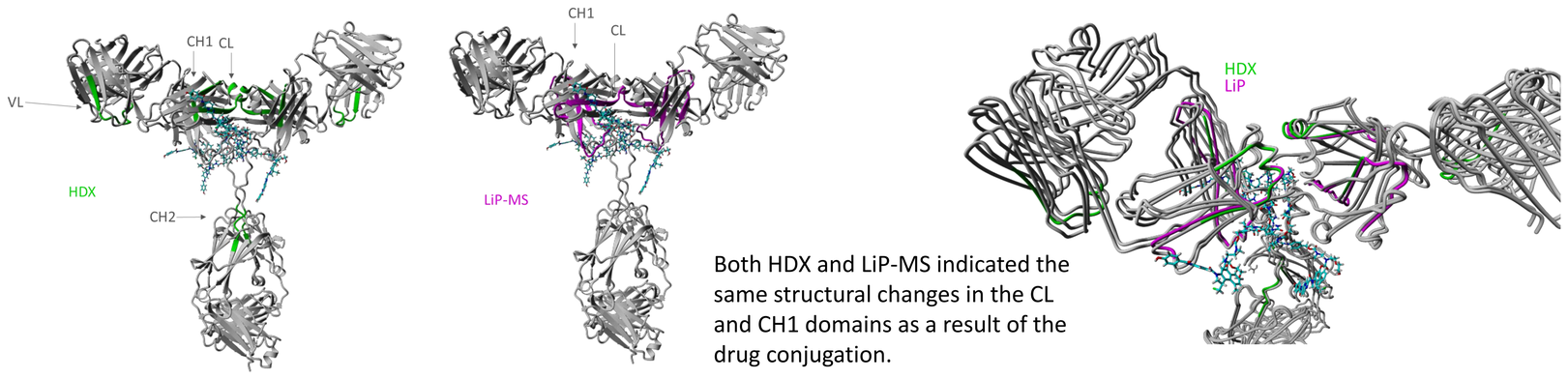


RESULTS



HDX MS showed very precisely regions that have undergone conformational changes upon linker-drug conjugation, as compared to the non-conjugated species, DAR0. The differences in solvent exposure between the different DAR species were small, but the acquired spatiotemporal deuterium uptake allowed us to locate precisely the affected sites in **VL, CL, CH1 and CH2 domains** with near amino acid resolution.

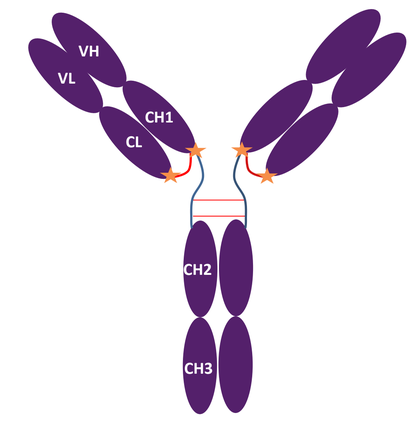




Both HDX and LiP-MS indicated the same structural changes in the CL and CH1 domains as a result of the drug conjugation.

Chymotryptic LiP-MS heat map

Peptide #	DAR2	DAR4	Domain
C1			
C2			
C3			
C4			
C5			
C6			VL
C7			
C8			
C9			
C10			
C11			
C12			
C13			
C14			
C15			
C16			
C17			
C18			CL
C19			
C20			
C21			
C22			
C23			
C24			
C25			
C26			
C27			
C28			
C29			
C30			
C31			
C32			
C33			
C34			VH
C35			
C36			
C37			
C38			
C39			
C40			
C41			
C42			
C43			
C44			
C45			
C46			
C47			
C48			CH1
C49			
C50			
C51			
C52			
C53			
C54			
C55			
C56			
C57			
C58			CH2
C59			
C60			
C61			
C62			
C63			
C64			
C65			
C66			
C67			
C68			CH3
C69			
C70			
C71			



A dual enzyme LiP-MS approach revealed regions in CL and CH1 domains that have become more accessible for proteolysis upon drug conjugation. These regions are located near the conjugation sites.

Tryptic LiP-MS heat map

Peptide #	DAR2	DAR4	Domain
T1			
T2			
T3			VL
T4			
T5			
T6			
T7			
T8			CL
T9			
T10			
T11			
T12			
T13			
T14			
T15			VH
T16			
T17			
T18			
T19			
T20			CH1
T21			
T22			
T23			
T24			
T25			
T26			
T27			
T28			CH2
T29			
T30			
T31			
T32			
T33			
T34			
T35			CH3

x17-fold

CONCLUSION

MS-based assays **confirm minor structural changes** resulting from drug conjugation and **locate** the exact sites in CH1 and CL domains
The **choice** of structural MS-method depends on the **purpose** of the investigation.

Take-home message

Both spectroscopic and MS-based methods are **sensitive** enough to detect subtle changes in protein HOS but they **differ** greatly in **resolution** and **specificity**.

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