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³ CNRS International Laboratory ARCHE, Art and Cultural Heritage: Natural Organic Polymers by Mass Spectrometry

Introduction

Mass spectrometry based methodologies have significantly improved molecular identification of complex materials in cultural heritage collections.

The challenge is now guided toward a better comprehension of proteins' **structural and conformational alterations** (protein interactions, formation of aggregations and networks) in artworks:

- ❖ Environment (e.g. different organic molecules or inorganic pigments)
- ❖ Storing conditions and ageing
- ❖ Conservation treatments

Currently no information is available on protein networks within paint layers, such as in tempera paintings.

Objectives and strategies

The presented research is intended to transcend the classical protein investigation by pursuing a better insight into the structural alterations of proteins in an artwork.

Innovative mass spectrometric strategies
(not been previously applied to ancient samples)

Hydrogen Deuterium Exchange combined with mass spectrometry (HDX-MS)

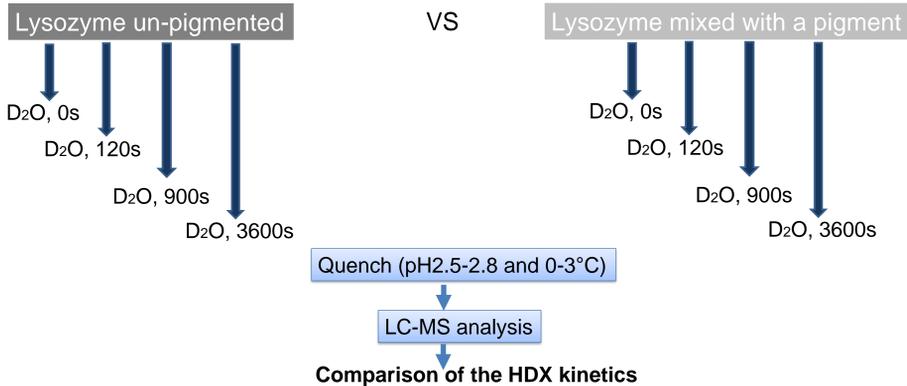
Effect of the pigment(s)
Measurement of the resulting protein conformation (and non covalent interactions between proteins and pigments)

Cross-linking investigation by mass spectrometry

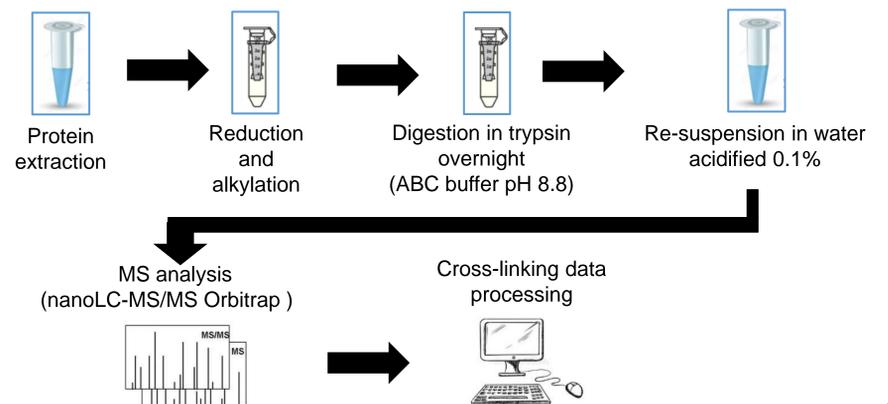
Information on protein network
Measurement of protein covalent interactions with other proteins

Workflow HDX-MS

Global approach (intact protein). On-exchange strategy with continuous labelling

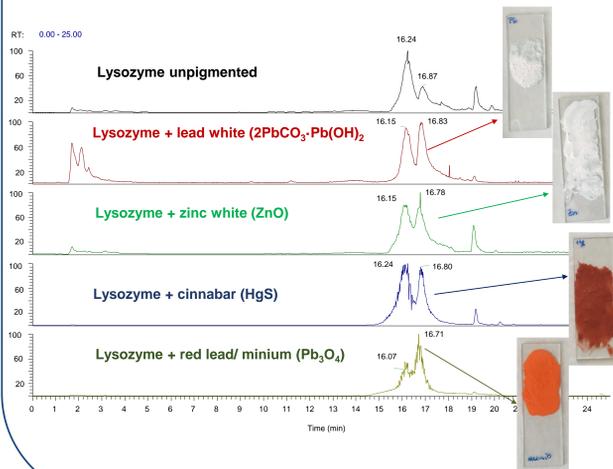


Workflow cross-links investigation



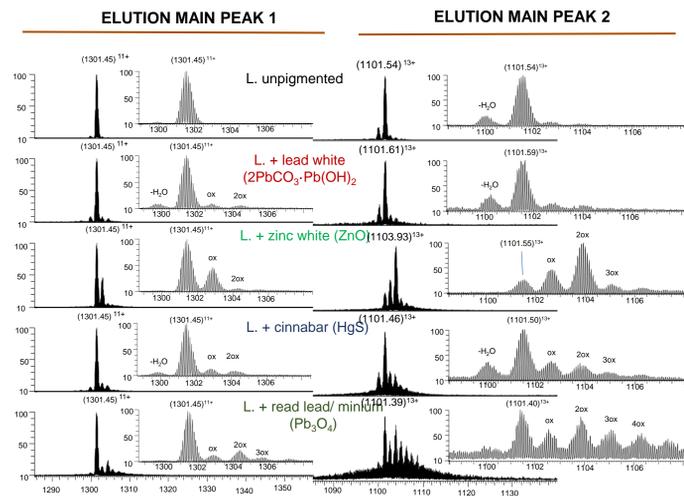
HDX-MS of painting models formulated with different pigments

Total ion current chromatograms



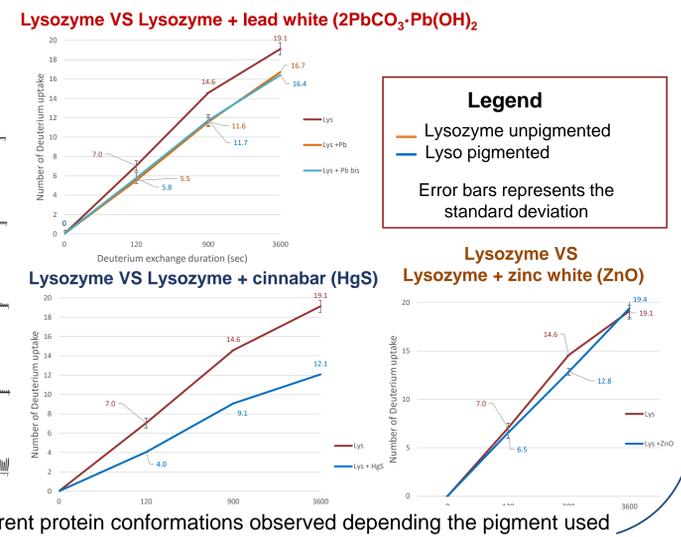
Distinct protein patterns identified depending the pigment used (here oxidation profiles shown)

HDX-MS at t0 (no exchange)



Example of fresh paintings following natural drying

Comparison of deuterium absorptions



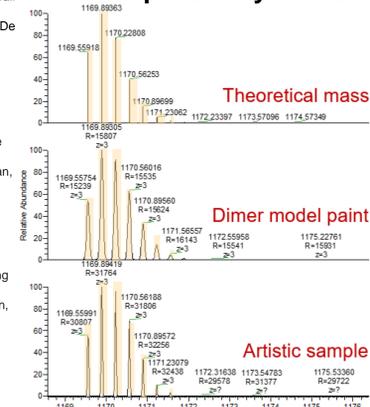
Crosslink formation in a sample with historic and artistic relevance

Few tens of µg of sample analysed

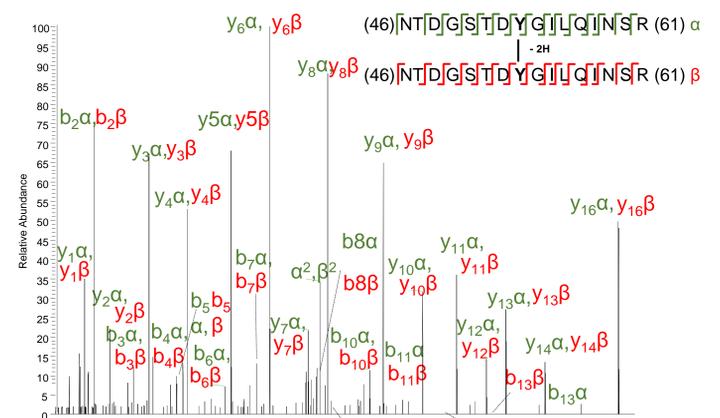
Crosslink type	Peptides
Y-Y	α NTDGSTDY53GILQINSR β GY23SLGNWVCAAK (NTDGSTDY53GILQINSR) ₂ (RHGLDNY20R) ₂
W-W	IVSDGNGMNAW108VAW 111R
H-K	α H14GLDNYR- β K1VFGFR



Example of dityrosine formation



Tandem mass spectrum showing an example of lysozyme crosslinked peptides



First structural characterizations of protein crosslinkings in historic artworks starting from few micrograms of samples

Conclusions and perspectives

Two innovative techniques not yet used in cultural heritage studies were developed and conducted combining hydrogen/deuterium exchange mass spectrometry (implementing an intact protein analysis mode) and cross-linking studies (bottom-up approach). Overall, both analyses resulted in a more comprehensive understanding of the conformational and structural modifications of proteins in formulation. The impact of the pigment type on the protein conformation is shown (example illustrated here: fresh paintings formulated with different various pigments, lead white, zinc white, cinnabar and read lead, after natural drying). Natural protein-protein crosslinks were also identified in historic paintings opening new insights on the study of protein networks in art material. A more in-depth investigation of proteins' structural alterations within a paint system is currently conducted by expanding the cross-linking formation research in relation to inorganic pigments and other organic molecules.