Hydrogen deuterium exchange mass spectrometry to elucidate reticulations, interactions and conformational changes of proteins in tempera paintings

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Mass spectrometry-based methodologies have significantly improved the molecular identification of complex materials in cultural heritage collections. Now, a critical challenge in this field to gain a better comprehension of the interactions of organic materials within the sample matrix related to their environment, ageing, or conservation treatments. There is no information available on protein networks within paint layers, such as in tempera paintings. The presented research is intended to advance the classical cultural heritage protein investigation by pursuing deeper insight into structural and conformational alterations in a proteinaceous binder during paint manufacture, drying and ageing. Not yet applied in cultural heritage studies, two innovative MS approaches, Hydrogen/Deuterium exchange (HDX) and cross-linking analysis, were used on both intact and digested proteins to study the protein networks within paint layers.

HDX technique studies protein conformation (protein folding) using hydrogen exchange between protein (amide hydrogens of the protein backbone) and its surrounding solvent. The rate of this exchange is dependent on the folded state of the protein and its dynamic. The HDX-MS experiments were carried out on paint mock-ups to explore molecular and structural changes before ageing; the mock-ups were mixtures of lysozyme (one dominant low molecular weight protein in egg proteins) with common inorganic pigments (lead white, zinc white, cinnabar, red lead). The first observation is the level of oxidation observed at the protein level dependently on the pigment used. HDX observations of the pigmented models suggest the variation of the protein conformation and/or the positioning of a pigment in the protein-pigment complex. These observations demonstrate that pigments and pigment type play a critical role in the molecular and structural alteration of proteinaceous binders already at the first stages of painting making and drying. With the addition of other paint additives, layering/mixing with other materials during use, and ageing over time, these modifications at the molecular level could have significant implications on the integrity and preservation of paint networks.

Complementary information on structural modifications was studied by investigating protein networking and cross-linking (i.e. covalent bonds between proteins and intra-proteins) via MS analysis. A data elaboration strategy capable of detecting and localising cross-links in an aged protein based artwork was developed to improve the representability of the results. The detection of cross linked peptides can enhance the findings of standard linear analyses by unveiling the protein fraction potentially undetected due to the reticulation. It can give a more detailed vision of the object's environmental conditions (i.e. basic pH, elevated exposure to photo-oxidation or presence of reactive compounds). The strategy was initially tested on the study of cross-links induced by oxidative stress in painting mock-ups starting from the very low sample amounts – few tens of micrograms. The method was then successfully applied to the

study of micro samples from several historic paintings from the Metropolitan Museum of Art collection.

The first molecular evidence of the impact of painting composition – particularly considering various pigments – on the protein structure during formulation, drying, and ageing will be presented. The initial hypothesis on protein network and breakdown in the studied historic paintings will be argued.