

Minimally invasive analysis, what can it tell us? The application of proteomics to ivory

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Structural proteins such as collagens are well preserved in mineralised tissues such as bones, teeth, and ivory, and can remain a source of taxonomic information long after the degradation of DNA. For this reason, the analysis of ancient proteins can be used to enable species identification of morphologically unidentifiable objects. We have optimised proteomic techniques to perform species identification on ivory and bone objects using minimally invasive procedures, and in this presentation, apply this method to the analysis of ivory objects from the collections of the Metropolitan Museum of Art.

Sampling was performed with 6µm microgrit polishing films, which were used to remove an extremely small amount of material from the surface of the object in sequential rubbings. In order to perform a successful analysis using such minimally invasive methods, it is necessary to miniaturise and simplify the sample preparation procedure. This is done by integrating different chemical treatments into a single or few steps, or using miniaturized analytical workflows. In addition to this, we have used data acquisition methods adapted to low sample amounts (Orbitrap Fusion Lumos, Thermo Fisher Scientific) and adapted our data processing steps to take into consideration the high heterogeneity of the collagen protein.

A further challenge is that many of the animals from which ivory originates have not had their proteomes fully sequenced, so the collagen sequences available on public databases have either been calculated from DNA sequences (e.g. African elephant), or are incomplete fragments (e.g. hippopotamus), modern ivory samples were obtained in order to build home-made proteomic databases.

In many cases we were able to identify several hundred peptides, including species-specific peptides, attributable to both collagen alpha1-(I) and alpha2-(I), from objects as old as 3200 years old. For example, in one case, a 957AA collagen alpha1-(I) fragment was identified with a coverage of 92% and 343 peptides. This means that we were also able to perform confident species identification based on several unique peptides that were confirmed with y and b fragment ions covering the sequences.

In this presentation we will: discuss how model samples were used to building home-made proteomic databases, discuss objects from the Metropolitan Museum of Art's collection which have been analysed, and introduce new methods that we are developing to further increase the amount of information we can obtain from such small sample amounts.

Key words: proteomics, minimally invasive procedure, ivory, collagen, species identification

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