Reaching trace level protein detection to study archaeological artefacts and museum objects: new proteomics methods based on high-resolution mass spectrometry

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Introduction

What?

Structural proteins such as collagens are well preserved in mineralised tissues such as bones, teeth, and ivory, and can remain a source of genetic information long after the degradation of DNA. For this reason, the analysis of ancient proteins is often used to enable species identification of morphologically unidentifiable bone fragments and to uncover new evolutionary relationships between hominid species.[1-3]

We are optimizing proteomic techniques to perform species identification on ivory and bone objects using minimally invasive procedures, specifically for use with museum and art objects.[4,5]

How?

In order to perform successful minimally invasive analysis, it is necessary to miniaturise and simplify both the sample preparation and data acquisition methods, which has already proven to improve recovery in the most challenging ancient samples.
In addition to this, the use of de novo sequencing of amino acid protein sequences will be necessary for the samples of interest, as they are expected to be derived from animals that have not had their genomes or proteomes fully sequenced. While de novo sequencing is commonly required in the field of palaeoproteomics, it has, as of yet, rarely been applied to cultural heritage objects to identify the species of origin.

Why?

Here, we focus on the analysis of collagen proteins in ancient bones, teeth and ivory. We are optimising the preparation of samples that have been collected using microgrit polishing films, as well as evaluating the effects of surface sampling techniques.

In the next steps, the results of this sample preparation optimisation will be applied to the case study of historic ivory and bone objects from the collections at The Metropolitan Museum of Art. Here, the goal of the research is to identify the species from which the objects originate, in order to better understand the context of the production and provenance of historic pieces.

Figure 1: Some of the objects that will be analysed in this study, in many cases it is not clear if they are made from bone or ivory due to the absence of the characteristic Shreger lines.

Methods

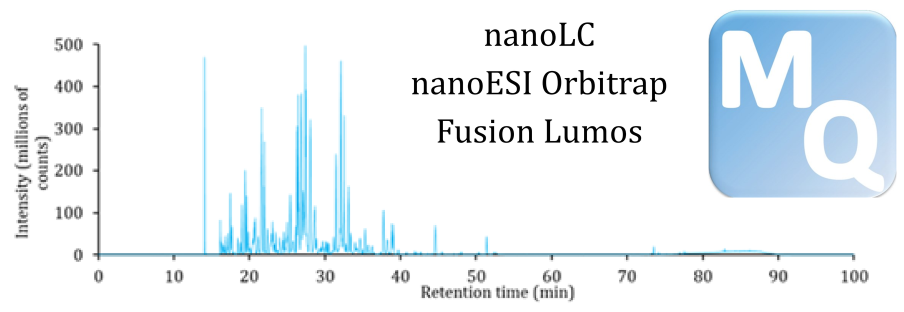
Sampling → Demineralisation → Extraction → Digestion → nanoLC-MS/MS → Preliminary data analysis → De Novo Sequencing → Manual confirmation

6 µm microgrit
Ivory
Bone
Teeth

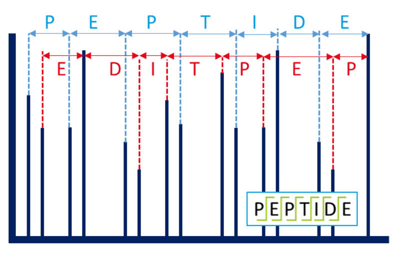
5% TFA

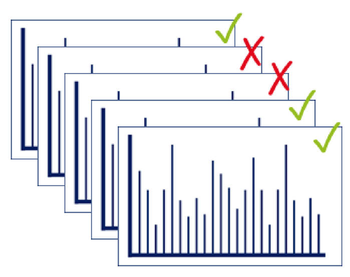
Reduction
Alkylation

Trypsin



nanoLC
nanoESI Orbitrap
Fusion Lumos





Results

1. Minimally invasive sampling significantly reduces the amount of material extracted from the sample

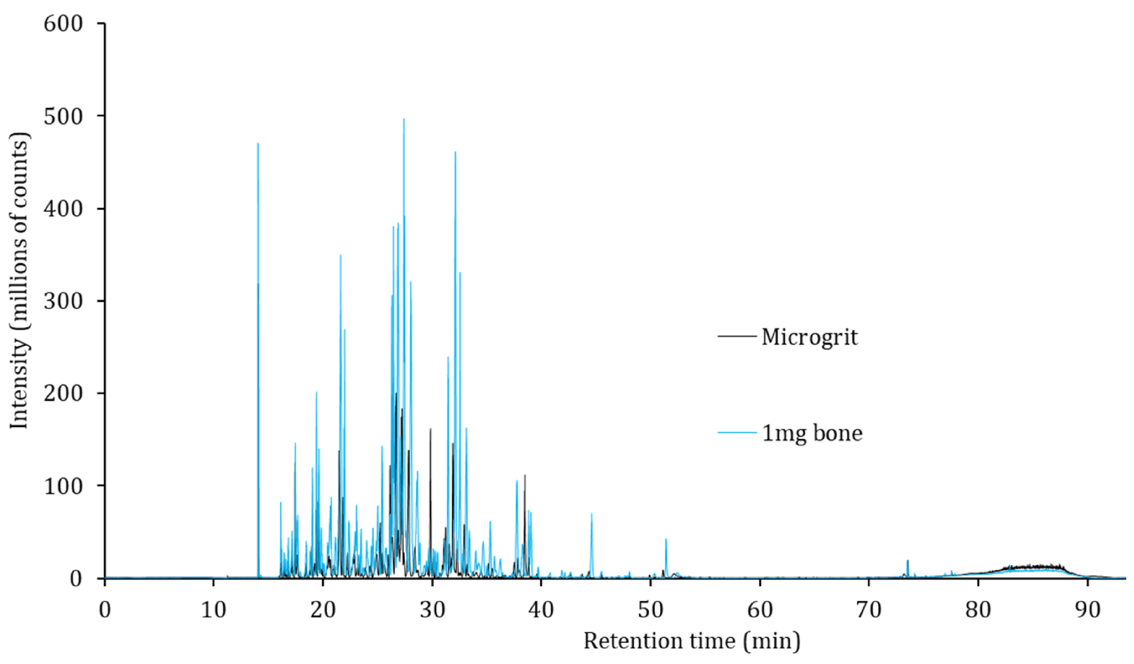


Figure 2: TIC chromatogram of tryptic peptides obtained from archaeological bone, using (a) 1mg of bone powder, and (b) a 6µm diamond microgrit (immeasurably small amount of sample).
Experimental: nanoLC, C18, 50cm, 2µm, Orbitrap Fusion Lumos

2. Increase in contamination when using surface sampling techniques, but consistent identification of key structural collagens

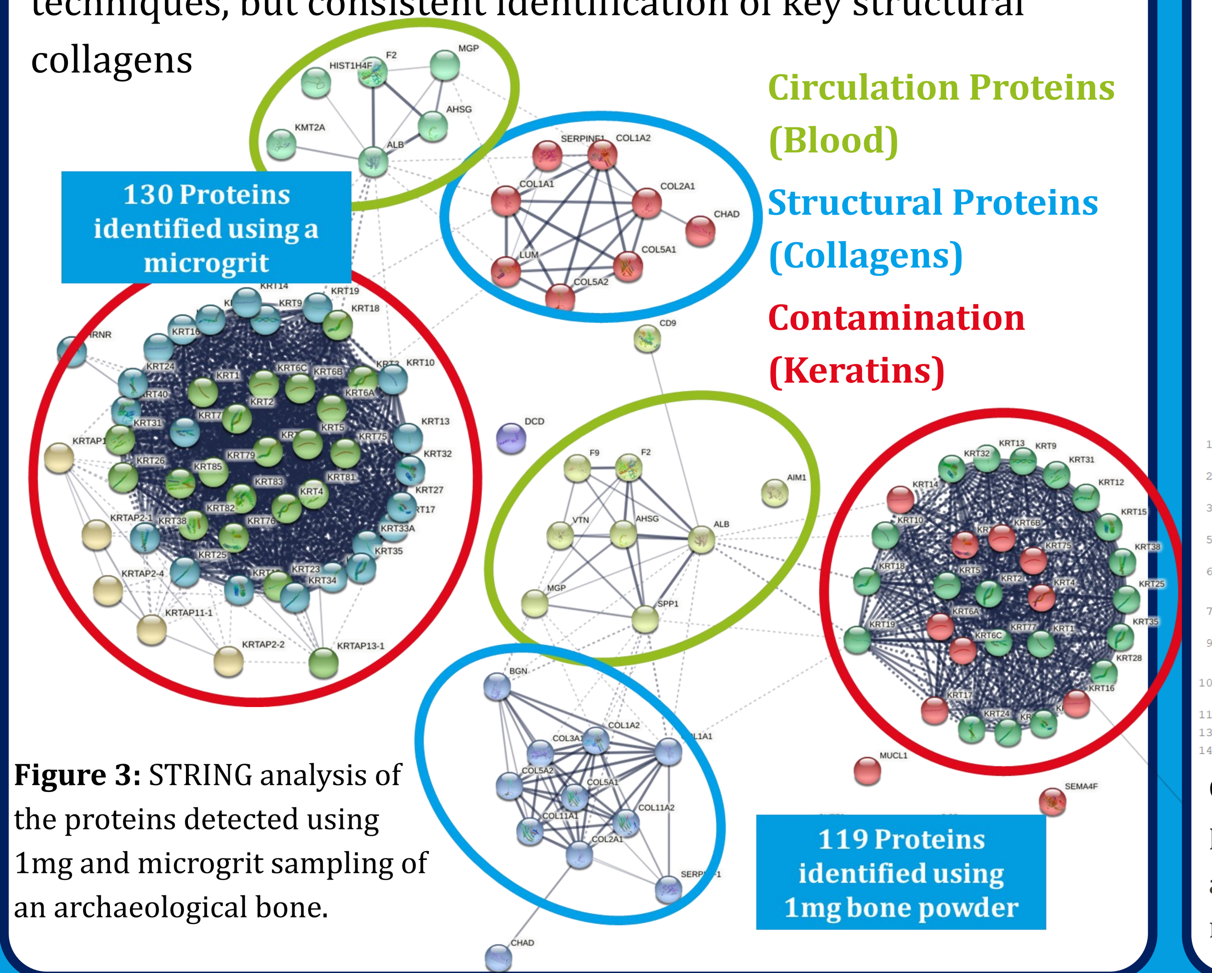


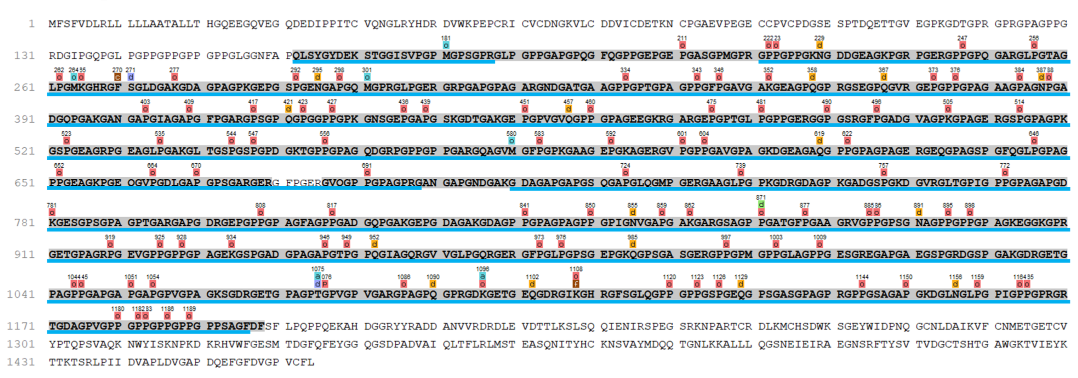
Figure 3: STRING analysis of the proteins detected using 1mg and microgrit sampling of an archaeological bone.

3. Comparable coverage of COL1A1 and COL1A2 proteins between experiments using powdered bone and microgrits.

Bone	% Coverage	
	COL1A1	COL1A2
1 mg	70	74
Microgrit	68	72

Tooth (Ivory model)	% Coverage	
	COL1A1	COL1A2
3 mg	60	69
Microgrit	41	27

Tables 1 and 2: % coverage of COL1A1 and COL1A2, the two most abundant proteins in mineralised tissues.



Collagen α(I)

Figure 4: A comparison of the sequence coverage obtained when performing a bottom-up proteomics analysis on 3mg of tooth and tooth sampled using a microgrit.

Current Work

- Alignment of collagen sequences of species of interest and close relatives of species that have not been referenced in protein databases (unconfirmed data extracted from the genome sequences).
- This alignment points out regions of interest in the proteins that will be used to discriminate between species.

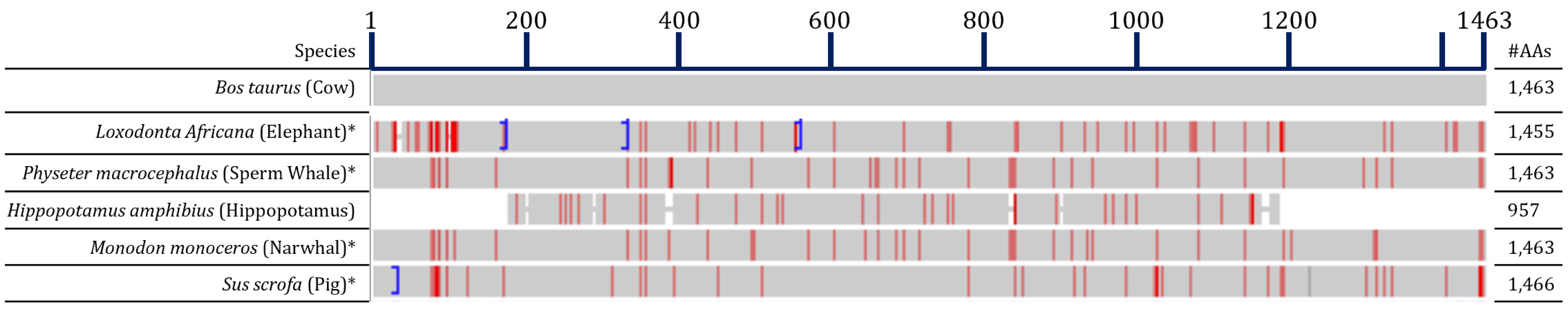
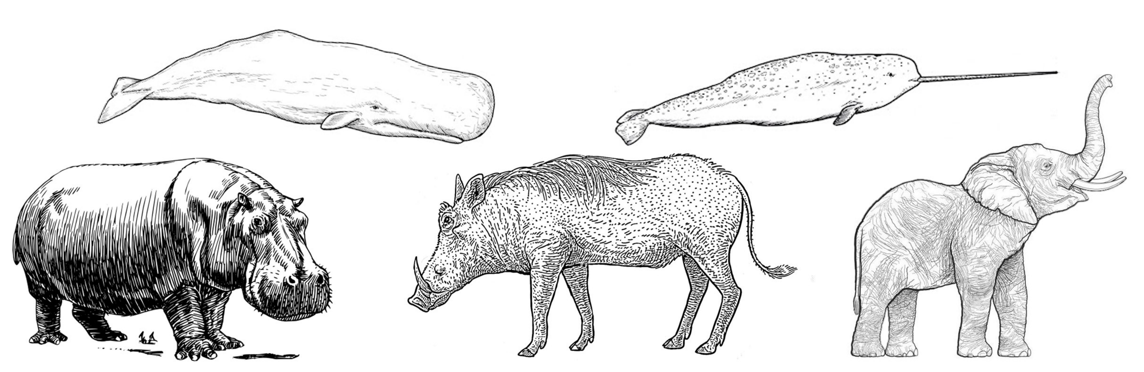


Figure 5: Sequence alignment of collagen alpha-1(I) of species of interest in this project, performed using the COBALT algorithm, available on NCBI. All species were compared against *Bos taurus*, red lines indicate where residues differ between the species and *Bos taurus*. *Indicates that the collagen sequences are predicted from DNA sequences.

Conclusions

- Challenging analysis due to the low protein content and hardness of ivory with the minimally invasive sampling.
- Preliminary results show very good COL1A1 coverage of minimally invasive analysis, current work is focusing on the repeatability of these results.
- Complimentary cleaning procedures are evaluated to reduce sample surface contamination.
- The results of this research will provide key insights into the history of objects in the Metropolitan Museum of Art's Egyptian and Medieval collections



References

[1] Coutu AN, Whitelaw G, le Roux P, Sealy J. Earliest Evidence for the Ivory Trade in Southern Africa: Isotopic and ZooMS Analysis of Seventh–Tenth Century ad Ivory from KwaZulu-Natal. African Archaeol Rev. 2016;33(4):411-435.; [2] Bradfield J, Forssman T, Spindler L, Antonites AR. Identifying the animal species used to manufacture bone arrowheads in South Africa. Archaeol Anthropol Sci. 2019;11:2419-2434; [3] Welker F, Ramos-Madrigal J, Gutenbrunner P, et al. The dental proteome of Homo antecessor. Nature. 2020;580(7802):235-238 ; [4] Pozzi F, Arslanoglu J, Galluzzi F, Tokarski C, Snyder R. Mixing, dipping, and fixing: the experimental drawing techniques of Thomas Gainsborough. Herit Sci. 2020;8(1):1-14; [5] Dallongeville S, Garnier N, Rolando C, Tokarski C. Proteins in Art, Archaeology, and Paleontology: From Detection to Identification. Chem Rev. 2016;116(1):2-79.

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