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FAUNAL SEX DETERMINATION BY PROTEOMICS, A VALUABLE PROSPECT IN BIOARCHAEOLOGY

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SCIENTIFIC BACKGROUND

- Bioarchaeology has highly benefited from the development of proteomics and mass spectrometry (MS) techniques [1], such as **Matrix-Assisted Laser Desorption Ionization** (MALDI) and high-resolution **Fourier Transform - Ion Cyclotron Resonance** (FT-ICR) MS. Zooarchaeological Mass Spectrometry (ZooMS) method is notably routinely used for taxa identification [2-4].
- Since the 2010's, methods using liquid chromatography-tandem MS (LC-MS/MS) have been implemented for sexing archaeological human teeth through the analysis of **amelogenin**, a protein contained in enamel and encoded by sex-chromosomes (AMEL-X, AMEL-Y) [5,6].
- The objective of this study is to assess the feasibility of using a **proteomics-based sexing method** for faunal species commonly found in Paleolithic sites in Eurasia, including **horse** (*Equus caballus*), **reindeer** (*Rangifer tarandus*), **hyena** (*Crocota crocuta*) and **cave bear** (*Ursus spelaeus*). Sexing faunal teeth remains would participate to deepen our understanding of Human-Fauna interactions.
- This approach is a relevant perspective to characterize archaeological faunal assemblages, as teeth are the most resistant material in the skeleton. Additionally, high-resolution MS offers the possibility to miniaturize samples size and support the rise of **micro-invasive protocols**.

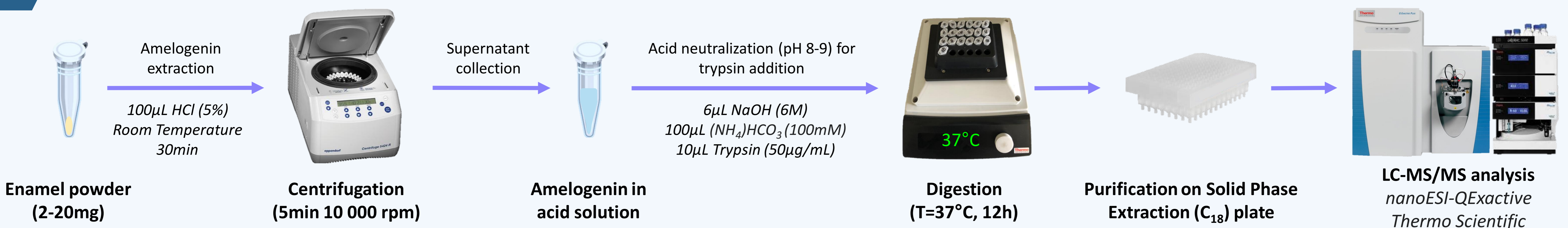
STUDY MATERIAL

This study includes archaeological and modern teeth of known-sex, determined through morphometric and genomic studies: horse (5 female teeth (F), 6 male teeth (M)), reindeer (4F, 5M), hyena (1F, 2M), and cave bear (1F, 1M). Samples of 2-20 mg enamel powder were taken for amelogenin extraction and analysis.



Figure 1: Archaeological teeth from the Paleolithic site of Tournal (France). (a) Male cave bear canine, (b) enamel micro-sampling of a reindeer molar. Magnification x15 of the sampling area.

CHEMICAL TREATMENT AND ANALYSIS



RESULTS – CASE STUDY: SEXING CAVE BEAR TEETH

Searching for sex-discriminating peptides in amelogenin sequences

Species	AMEL-X	AMEL-Y
<i>Ursus arctos</i>	MGTWILFACLLGAAAFAMPLPPHPGHPGYINFSYENAHFQAINTIDRTASVLTPLKQYQNMIRHPYPSYGYEP	MGTWILFACLLGAAAFAMPLPPHPGHPGYINFSYENAHFQAINTIDRTASVLTPLKQYQNMIRHPYPSYGYEP
<i>Ursus maritimus</i>	MGTWILFACLLGAAAFAMPLPPHPGHPGYINFSYENAHFQAINTIDRTASVLTPLKQYQNMIRHPYPSYGYEP	MGTWILFACLLGAAAFAMPLPPHPGHPGYINFSYENAHFQAINTIDRTASVLTPLKQYQNMIRHPYPSYGYEP
<i>Ursus americanus</i>	MGTWILFACLLGAAAFAMPLPPHPGHPGYINFSYENAHFQAINTIDRTASVLTPLKQYQNMIRHPYPSYGYEP	MGTWILFACLLGAAAFAMPLPPHPGHPGYINFSYENAHFQAINTIDRTASVLTPLKQYQNMIRHPYPSYGYEP

Figure 2: Amelogenin sequences (AMEL-X, AMEL-Y) of *Ursus arctos*, *Ursus maritimus* and *Ursus americanus* [7,8]. Red frames: Amino acids showing sex-discrimination. Peptide sequences are obtained through the translation of nucleotides. All data are exported from NCBI (National Center for Biotechnology Information). NCBI data references, from top to bottom: XP_044247823.1, XP_008694624.2, XP_045661043.1, XP_026346480.2, XP_008708736.2, AAO60680.1, XP_045634868.1. Sequences are aligned using Uniprot tool.

Matching sample peptides to amelogenin sequences

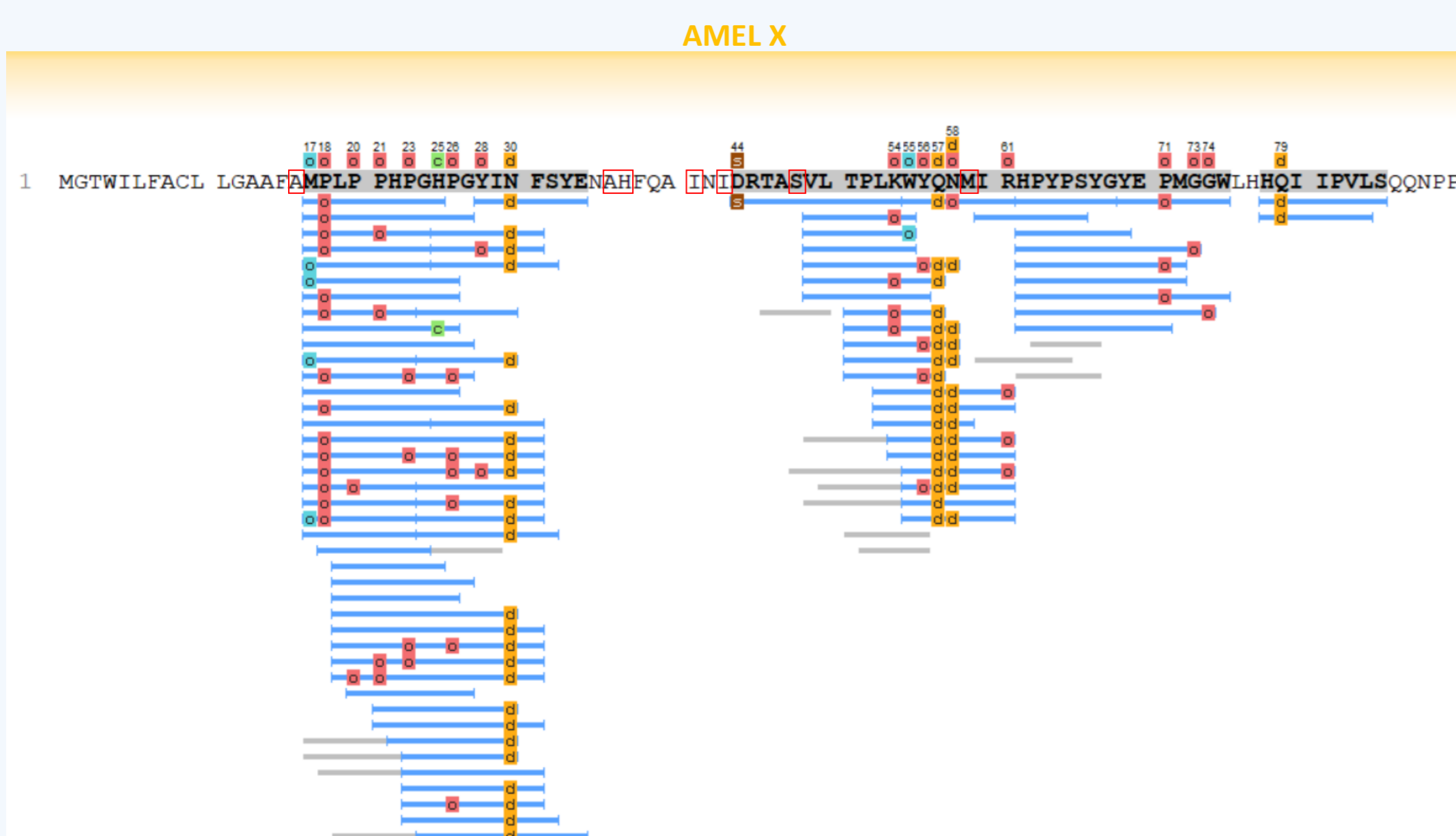


Figure 3: Peptides corresponding to portions of the amelogenin protein extracted from a male cave bear tooth are reported (blue bars) and aligned against an AMEL-X reference sequence corresponding to brown bear (*Ursus arctos*) using PEAKS Studio12 software. Chemical degradations occurring on peptides are indicated with letters: d: deamidation (+0,98), o: oxidation (+15,99), s: sodium adduct (+21,98), c: carbamidomethylation (+57,02). Red frames: Sex-discriminating amino acids for brown bear AMEL-X sequence.

Fragmentation mass spectrum of two discriminating peptides

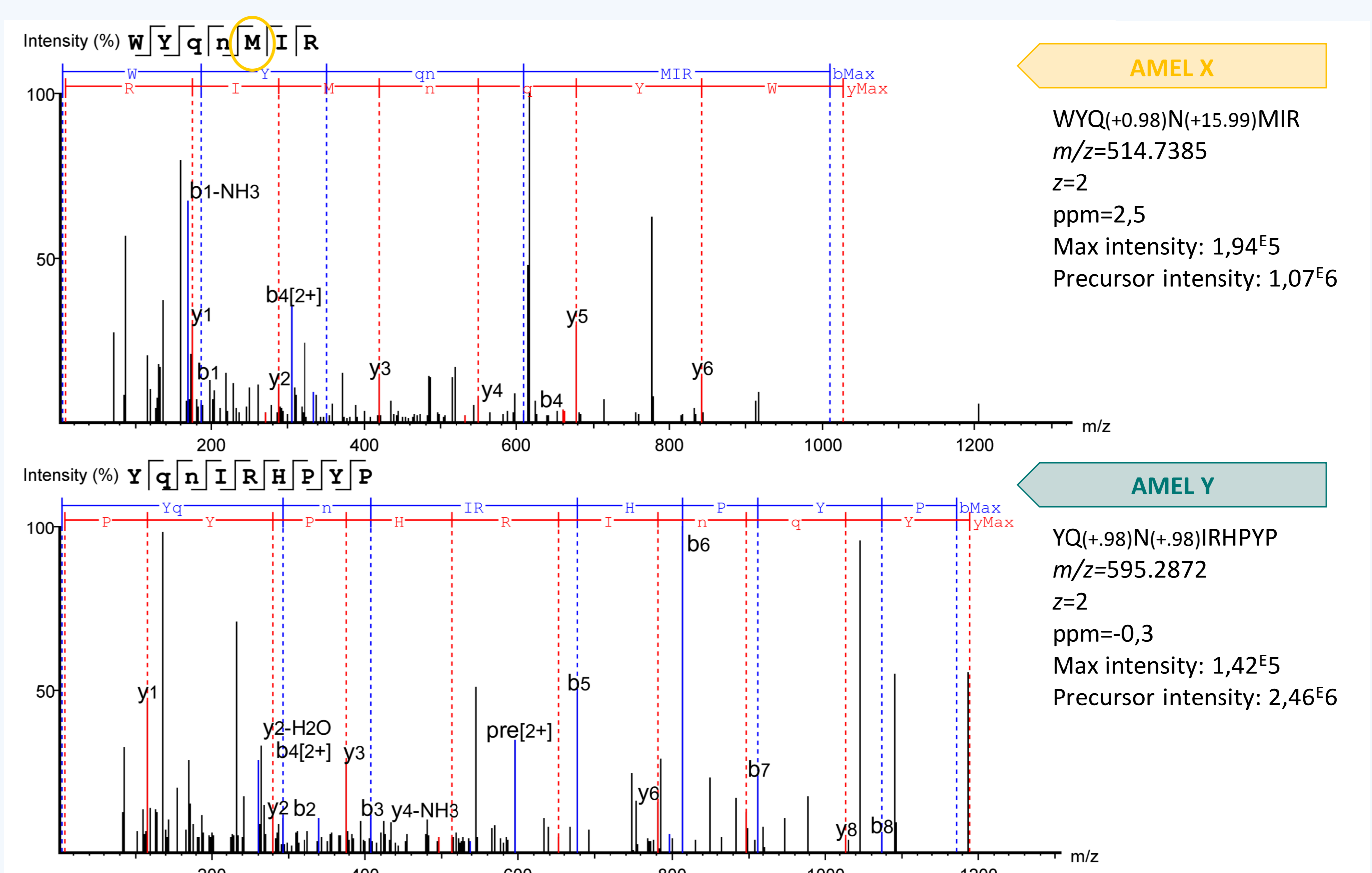


Figure 4: Fragmentation mass spectrum of two discriminant peptides obtained on a male cave bear enamel sample, using LC-MS/MS, hybrid quadrupole-Orbitrap (nanoESI-QExactive Thermo Scientific).

CONCLUSIONS

- This study has proven the feasibility of sexing Paleolithic cave bear teeth by proteomics, using known-sex samples and reference amelogenin sequences of genus *Ursus*.
- The data analysis for the sexing of horse (*Equus caballus*), reindeer (*Rangifer tarandus*), and hyena (*Crocota crocuta*) is ongoing and results will be released soon.
- Proteomic sexing of faunal teeth appears to be a promising prospect in bioarchaeology. This will undoubtedly participate to enhance our understanding of faunal evolution and metabolism as well as Human subsistence strategies and Human-carnivore interactions or competition.

BIBLIOGRAPHY

- Warinner, et al. Chem. Rev. 122, 13401–13446 (2022), <https://doi.org/10.1021/acs.chemrev.1c00703>
- Bray, et al. Anal. Chem. 95, 7422–7432 (2023), <https://doi.org/10.1021/acs.analchem.2c03301>
- Richter, et al. PNAS. 119, e2109323119 (2022), <https://doi.org/10.1073/pnas.2109323119>
- Devrière, et al. PNAS. 114, 10606–10611 (2017), <https://doi.org/10.1073/pnas.1709235114>
- Stewart, et al. RSC Adv. 6, 61673–61679 (2016), <https://doi.org/10.1039/C6RA05120K>
- Gwenaëlle Goude, et al. 30th EAA Annual Meeting (Rome, Italie), Session 931, (2024), <https://hal.science/hal-04690550>
- Carmichael et al. J. Mammal. 86, 160–169 (2005), [https://doi.org/10.1644/1545-1542\(2005\)086<0160:GOPPI>2.0.CO;2](https://doi.org/10.1644/1545-1542(2005)086<0160:GOPPI>2.0.CO;2)
- Pagès et al. Conserv. Genet. 10, 897–907 (2009), <https://doi.org/10.1007/s10592-008-9650-x>

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