

## Ancient tooth proteomes

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Teeth are a remarkable source of information in paleoanthropological research. Investigation of ancient dental proteomes has the potential to provide complementary biomolecular data such as sex and taxonomic determination, phenotype and disease marker identification, or diet composition, that are essential to understand morphostructural signatures of fossils remains and to get insight on human phylogeny and ancient physiopathology.

In the frame of setting up paleoproteomic approaches, human teeth from different archeological periods (present-day to Neolithic) and geographical areas have been compared and analyzed by online nanoflow liquid chromatography coupled to tandem mass spectrometry (nanoLC-MS/MS) using Q Exactive™ Plus and Orbitrap Fusion™ Tribrid™ mass spectrometers. At each step, protocols and procedures have been adapted to the particular case of ancient samples, i.e. low content proteins and contamination by modern or environmental materials, by careful manipulation in cleanrooms and by insertion of appropriate blank controls. The degraded and modified proteins are another major issue. Therefore, a protein identification workflow using iterative searches with home-build protein databases and Proteome Discoverer™ software has been optimized to take into account the high events of modifications (deamination, oxidation, carbamylation) and missed cleavages as well as the non-tryptic and the variant peptides containing diagenetic amino-acid substitutions.

Among the proteins identified in dental tissues, some of them such as amelogenin, ameloblastin, enamelin, amelotin, dentin sialophosphoprotein, are very specific to enamel or dentin, ensuring therefore the reliability of the analyzed samples. In addition, the detection of the two forms of amelogenin encoded by the chromosomes X (AMELX) and Y (AMELY) potentially offers the opportunity to characterize male specimens, an essential information for paleoanthropologists when confronted to sexual dimorphism. For this purpose, PRM-based targeted mass spectrometry approaches has been implemented to systematically and precisely detect and identify the sex discrimination peptides of amelogenin as well as quantify them.

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