

# Analysis of Human Neolithic Tooth Proteomes

Carine Froment<sup>1</sup>, Mathilde Hourset<sup>2,3</sup>, Nancy Saenz<sup>2</sup>, Claire Willmann<sup>2</sup>, Emmanuelle Mouton-Barbosa<sup>1</sup>, Clément Zanolli<sup>2</sup>, Rémi Esclassan<sup>2,3</sup>, Catherine Thèves<sup>2</sup>, Odile Burlet-Schiltz<sup>1</sup> and Catherine Mollereau<sup>2</sup>

<sup>1</sup> *Institut de Pharmacologie et Biologie Structurale (IPBS), UMR5089 CNRS - Université de Toulouse, 205 route de Narbonne, 31077 Toulouse, France*

<sup>2</sup> *Laboratoire d'Anthropologie Moléculaire et Imagerie de Synthèse (AMIS), UMR5288 CNRS - Université de Toulouse, 37 allées Jules Guesde, 31073 Toulouse, France*

<sup>3</sup> *Faculté de chirurgie dentaire - Université de Toulouse, 3 Chemin des Maraîchers, Toulouse, France.*

Investigation of ancient tooth proteomes is able to provide valuable data such as sex and taxonomic determination, phenotype and disease identification, or diet composition, that are essential to understand morphostructural signatures of fossils remains and to get insight on human phylogeny, past life and ancient physiopathology. We have set up a dedicated paleoproteomic strategy combining shotgun MS-based approaches for the investigation of dental proteomes and targeted MS-based approaches for sex determination.

5500-year old human teeth from the Neolithic site of Mont Aimé (France) have been analyzed by online 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, characterized by low and degraded protein content, diagenetic or evolutionary modification, and presence of modern or environmental contaminants. This included a careful manipulation in cleanrooms and the insertion of appropriate blank controls and column washing procedures. In addition, a protein identification workflow using iterative searches with home-build protein databases and Proteome Discoverer™ software has been optimized to take into account several modifications (deamidation, oxidation, carbamoylation), missed cleavages, non-tryptic peptides, and archaic variant peptides with amino-acid substitutions. A PRM-based targeted MS approach has been implemented to detect, identify, and quantify systematically and precisely the sex specific peptides of amelogenin.

The innovative iterative search workflow allowed to identify a large number (500-1400) of proteins in samples, while only 4-32 proteins in negative controls (essentially collagens, keratins and serum albumin), indicating a low level of contamination during extraction and MS analysis. A large part of proteins were identified in the non-tryptic search mode. The two forms of amelogenin, AMELX and AMELY were detected, as well as archaic variants with taxonomic interest.

Finding a complex proteome in 5000 year old teeth may be explained by a permanent regeneration of the dentine tissue induced by the damages caused by the roughly grinded regimen of the Neolithic populations at the early stage of agriculture. The ability to detect sex-specific AMEL peptides by targeted MS is promising for sex determination of ambiguous teeth fossils.

**Keywords:** Paleoproteomics, Database iterative searches, Targeted MS, Ancient Teeth