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## **Preliminary palaeoproteomic insights into the Plio-Pleistocene australopiths from South Africa**

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## Preliminary palaeoproteomic insights into the Plio-Pleistocene australopiths from South Africa

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Palaeoproteomics allows for the recovery of genetic information beyond the limits of ancient DNA preservation. Dental enamel proteins older than 900 thousand years from temperate and subtropical areas can be phylogenetically informative about phylogeny, as recently demonstrated by the analysis of fossil teeth from *Gigantopithecus blacki* (1.9 Ma) [1]. Recent work on *Homo antecessor* (900 ka) [2] has demonstrated the viability of applying these approaches to ancient fossil hominins.

Here we recovered dental enamel proteins from australopith remains from South Africa: in particular, four *Paranthropus robustus* specimens from Swartkrans, dated to 2.2-1.8 Ma, and 1 specimen of *Australopithecus africanus* from Sterkfontein, dated to 2.8-2.2 Ma. This enabled us to determine the sex of these individuals and learn about their phylogenetic placement relative to present-day and ancient hominids.

We employed a digestion-free enamel extraction protocol [3] to recover ancient hominin proteins from the South African specimens SK 830, SK 835, SK 850, SK 14132, and Sts 63, and leveraged tandem mass spectrometry for deep sequencing. To confidently assign the retrieved MS/MS spectra to peptide sequences, we used reference database-dependent and open searches, using MaxQuant [4] and Peaks [5] software. The reference database included enamel and dentine protein sequences from publicly available repositories (Uniprot and NCBI) taxonomically restricted to hominids. For those taxa whose enamel and dentine protein sequences were not available, we translated protein sequences from publicly available genomic archives

Our results show that it is possible to retrieve enamel proteins from these ancient South African hominins. This is an encouraging step forward in ancient protein studies and is the first ancient proteome recovered from African Plio-Pleistocene fossils. Specimens SK 835, SK 850 and Sts 63 are identified as biologically male individuals. This opens the possibility of reassessing our understanding of sexual dimorphism in these and other early hominins and differentiating variation due to dimorphism from that resulting from taxonomic distinctiveness. Although the recovered enamel proteome is small, we also identified phylogenetically informative single amino acid polymorphisms. Future work will focus on expanding our sample, to better understand variation within and between early hominin taxa in Africa.

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