Export permits

Please note an export permit must be linked to an object or site that has to be created on SAHRIS! If the object/site you want to work on has not been created yet, you would need to do so. Thanks!

The proposal should include (you can fill these in below):

- **a list of participants (name, affiliation, phone no, email addresses) and how they are involved;**

  Prof. Kirsty Penkman (PI on the Wisdom Teeth grant), University of York, York, YO10 5DD, UK (kirsty.penkman@york.ac.uk) - +44 1904 322574 – interpretation of results

  Dr Marc Dickinson, University of York, YO10 5DD, York, UK (marc.dickinson@york.ac.uk) – +44 7946807980 – preparation and analysis of samples, and interpretation of results

  Prof. José Braga, University of Toulouse, UMR 5288 CNRS, Toulouse, France (jose.braga@univ-tlse3.fr) + 33 6 37 78 22 32 – Interpretation of results

- **the name and address of the facility, including address, it is being investigated at;**
  NEaar lab, Chemistry department, University o York, York, YO10 5DD, UK

- **name and address of the museum/university department that currently hosts the object**;

  Evolutionary Studies Institute, University of the Witwatersrand, Private Bag 3. Wits 2050, South Africa. [+27-11] 717-6690/6682.

- **names of the responsible person(s) during transport and while the fossil is at the facility;**
  Prof. Kirsty Penkman, Dr Marc Dickinson and Prof. José Braga

- **the period/time frame during which the fossil(s) will be outside the country;**

- **detailed information on the fossil(s), especially as it is a "unique" specimen;**

  A total of 17 bovid or proboscidean specimens recovered from the site of Kromdraai within its Unit O, Unit P or a Unit stratigraphically younger (Unit S>) and listed as follows:

  UNIT.O_KW.10876
  UNIT.O?_KW.10107
  UNIT.P_KW.7621b
  UNIT.P_KW.7659
  UNIT.P_KW.9021
  UNIT.P_KW.9681
  UNIT.P_KW.9702
  UNIT.P_KW.9745
  UNIT.P_KW.9765
  UNIT.P_KW.9923
  UNIT.P_KW.9982
  UNIT.P_KW.10005b
  UNIT.P_KW.10066
  UNIT.P_KW.10449
  UNIT.P_KW.10645
  UNIT.P_KW.10824
  UNIT.S>_KW.10204

- **detailed information on the research project behind it & methodology including expected outcomes (i.e., the reason for export);**

This research is been conducted as part of the Wisdom Teeth project (https://sites.google.com/york.ac.uk/wisdom-teeth/home) which aims to make use of the recent breakthrough made in using protein degradation to date tooth enamel. Applying it to regions where the palaeoenvironmental record can help us understand the sensitivity of Africa's mammalian fauna to climate change. In doing so, it will provide a new, more accurately dated record for the African Pleistocene and Late Pliocene, unlocking insights into our own evolutionary history.

Amino acids are the building blocks of proteins, which are found in all living tissues and can be preserved in fossil biominerals such enamel or shells. Intracrystalline protein degradation (IcPD) dating relies on the predictable breakdown of proteins and amino acids within the closed system environment of a given biomineral, to give a direct estimate of the age. IcPD analysis of molluscan material from Europe has shown that IcPD dating covers at least the last 2.5 Ma, and thus is applicable to the whole of the Quaternary Period (Penkman et al., 2011; 2013). Additionally, recent IcPD analysis of teeth from a range of genera from European deposits suggests enamel IcPD may be able to date material further back in time, potentially into the Pliocene (Dickinson et al., 2019; Cappellini et al., 2019; Welker et al., 2020). However, the range for which it is applicable is highly dependent of the temperature history of the samples and thus in warmer climates the applicable dating range may be shorter.

Most amino acids can exist in 2 forms which are non-superimposable mirror images of each other (Figure), designated left-handed (*laevo,* L-form) and right-handed (*dextro,* D-form). In living organisms, proteins are almost exclusively made from the L-form. However after death, a spontaneous reaction (called racemization) starts to occur. This leads to a progressively increasing proportion of the D-form in direct relation to the time elapsed, until the D and L forms are present in equal quantities. Depending on the amino acid, this process can take thousands or millions of years and therefore is applicable over Quaternary timescales. The extent of amino acid racemisation (AAR) in a sample is recorded as a D/L value, and its age can thus be determined based on (a) which amino acid it is, (b) the species being analysed, and (c) a baseline reference framework of comparative data from independently dated sites (an aminostratigraphy). There are several measures of protein degradation that can be used to estimate the age of a fossil, such as extent of peptide hydrolysis and changes in amino acid composition but the most time sensitive process of degradation used for age estimation is AAR.

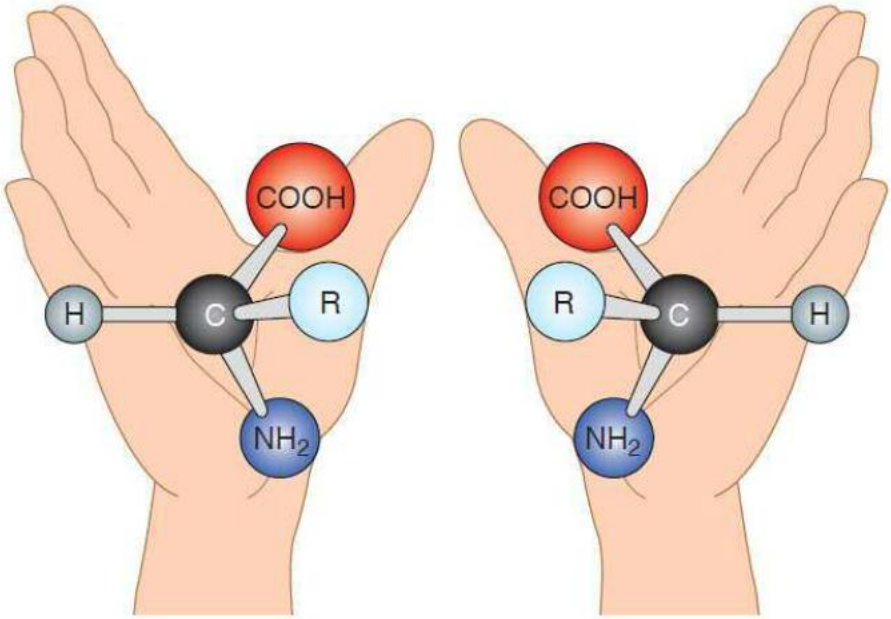

Figure 1. Most amino acids have no plane of symmetry, just like hands, so their mirror images are non-superimposable and therefore distinct from each other. The breakdown of left-handed molecules to the right-handed form over time provides a mechanism for estimating age of fossil material.

Protein degradation consists of a series of chemical reactions that are dependent not only on time, but also on environmental factors (e.g. pH, availability of water), which can confound the time signal. These difficulties in AAR's early applications have led to a focus on analysing 'closed-system' protein from fossil samples (Towe, 1980; Brooks et al., 1990), where the fraction of protein analysed is physically or chemically shielded from the environment and the difficulties associated with contamination, leaching and environmental factors are circumvented (Penkman et al., 2008). Tooth enamel has been found to contain a fraction of intra-crystalline amino acids that exhibit closed system behaviour, meaning that the extent of AAR within this fraction is solely time and temperature dependent (Penkman *et al*., 2008; Dickinson et al., 2019).

The rate of breakdown towards D/L equilibrium in the intra-crystalline fraction is still affected by temperature, so comparative frameworks need to be applied from regions with a broadly similar temperature history, which is currently being developed for South Africa through a NERC-funded Wisdom Teeth Project. Analyses are routinely undertaken on the total hydrolysable amino acid fraction (THAA, which includes both free and peptide-bound amino acids), and often also on the free amino acid fraction (FAA, produced by natural hydrolysis).

The method involves removing a small sample of enamel (~30-50 mg) and then removing all other dental components (e.g. dentine) from the sample. This is done using a precision drill with a small abrasive drill bit. The drill should be kept on the slowest setting to reduce the impact of the drilling on the specimen. The samples are then powder using an agate pestle and mortar and bleached to remove all sources of external contamination and to isolate an intracrystalline fraction (Dickinson et al., 2019). Two subsamples are then taken from the bleached material: one is used to analysed the free amino acid (FAA) content, the other the total hydrolysable amino acid (THAA) content. The THAA fraction is treated with strong acid to hydrolyse the peptides and proteins. After the inorganic mineral has been removed, the samples are analysed by RP-HPLC to determine the amino acid composition and ratio of D and L amino acid.

It is expected that the project will generate relative age estimates for the material analysed and that these results will be published alongside other data from material from South Africa. Upon publication, amino acid data are now being archived by NOAA and are freely available at

http://www.ncdc.noaa.gov/paleo/aar.html.

- **the written confirmation of the institution that currently hosts the object that the object may be used as proposed and be returned in good condition;**

- **should there be any damage/destructive analysis (e.g., coating for higher resolution) undertaken, this needs to be stated in detail;**
  A small amount of enamel will be removed from each of the specimens (~30-50 mg). The sample area will be chosen to minimize the impact on the sample and does not need to be from a specific area. Therefore, areas that have already fragmented are often preferable.

- **Statement why this study cannot be done in South Africa.**
  The analysis of proteins/amino acids for this purpose needs to be conducted in an amino acid clean lab to prevent contamination of the samples. It is therefore not possible to conduct the analysis in South Africa.

Applicant (name and affiliation): this is usually the museum curator!

Dr Bernhard Zipfel, Evolutionary Studies Institute, University of the Witwatersrand

Applied for (principal researcher):
Dr Marc Dickinson, University of York, YO10 5DD, York, UK (marc.dickinson@york.ac.uk)

Participants with affiliations, email addresses, phone numbers (& their role):

Pr José Braga, University of Toulouse, UMR 5288 CNRS, Toulouse, France
- Interpretation of results

Prof. Kirsty Penkman (PI on the Wisdom Teeth grant), University of York, York, YO10 5DD, UK (kirsty.penkman@york.ac.uk) - +44 1904 322574 – interpretation of results

Institution incl. address that currently hosts the object:

Evolutionary Studies Institute, University of the Witwatersrand, Private Bag 3. Wits 2050, South Africa. [+27-11] 717-6690/6682.

Facility incl. address at which the experiment will be done:
NEaar lab, Chemistry department, University of York, York, YO10 5DD, UK

Table of objects or upload file:

Time frame:

Aim/rationale:
Establishing an accurate chronology is imperative to understand mammalian (including hominin) evolution (taxonomy and phylogeny), and for decoding potential relationships to biogeography, environmental change and global climate. Most of the fossils targeted in this study were recovered in situ from Unit P at Kromdraai. Unit P represents one of the two newly recognized hominin-bearing sedimentary deposits that have been identified during recent fieldwork at this site (Braga et al., 2017).

Unit P was previously identified as “Member 2” when it was considered as “sterile” (Vrba, 1981). It was still named “Member 2” in the most recent description of the stratigraphy of Kromdraai (Bruxelles et al., 2016) and when the discovery of new fossil hominin specimens was subsequently announced (Braga et al., 2017). The term “Unit” was recently used to designate Unit P and any other depositional interval distinguishable above and below at the scale of the Kromdraai site (Ngoloyi et al., 2020). This change was decided to follow the conventional lithostratigraphic terminology used by the International Commission on Stratigraphy (stratigraphy.org), in which a “Member” does not designate “the smallest formal unit in the hierarchy of sedimentary lithostratigraphic units”. The stratigraphic sequence at Kromdraai starts with Unit A which contains no fossils and corresponds to the “Stony breccia” as described in Brain (1958), and “Member 1” as described in Vrba (1981), Partridge (1982) and Bruxelles et al. (2016). Unit P is stratigraphically older than the immediately overlying sedimentary deposits of “Member 3” - as described in Vrba (1981), Partridge (1982) and Bruxelles et al. (2016) - and subsequently subdivided by Bruxelles et al. (2016) into “Member 3” (now renamed Unit Q) and its overlying “Submember 4.1” (now renamed Unit R). A few hominin specimens were recovered within Units Q-R either in situ or ex-situ (Braga et al., 2017). Before the present study, only two non-dental diagnostic cranial remains were available in the hominin assemblage from Kromdraai (Braga et al., 2013): TM 1517, the unprovenanced type specimen of *P. robustus* (Broom, 1938a) and KB 6067, an isolated left petrous bone (Braga et al., 2013). As detailed in Braga et al. (2013), whereas KB 6067 can be tied to Units Q-R, TM 1517 comes from “significantly younger layers”. Therefore, *Paranthropus* specimens from Unit P are stratigraphically older than both TM 1517 and KB 6067 specimens. The spatial patterning of the fossil hominin and non-hominin assemblage recovered in situ from Unit P until 2017 (Ngoloyi et al., 2020) describes four main clusters interpreted as either areas of higher density of fossils, or as accumulations resulting from particular processes (e.g., distinct entrances). The stratigraphically older Unit O represents a previously unrealised fossiliferous deposits referred to as “Member 1” in Braga et al. (2017). Unpublished Australopithecus specimens and faunal material have been recently recovered in situ from Unit O.

Therefore, the Kromdraai site offers the opportunity to trace, for the first time within one single stratigraphic sequence in South Africa, the paleoecological changes associated with the evolution (i) within the *Paranthropus* lineage (i.e., between Units Q/R and Unit P); (ii) between *Australopithecus* and *Paranthropus*/*Homo* (i.e. through the two oldest stratigraphic Units O and P, unpublished data [Figure 1]), on a relative time scale that overlaps on the Pliocene-Pleistocene boundary and documents. Units O and P have high numbers of fossil teeth representing carnivores, cercopithecoids and bovids, and thus the site is ideal for the recently developed approach of enamel intra-crystalline protein degradation (IcPD) dating (Dickinson et al., 2019). Through building relative chronologies based on the mammalian taxa associated with the hominin specimens, it is hoped that enamel IcPD dating will be able to create a clearer understanding of the chronology of the site, and therefore aid in interpretation of the context of these important hominin remains.

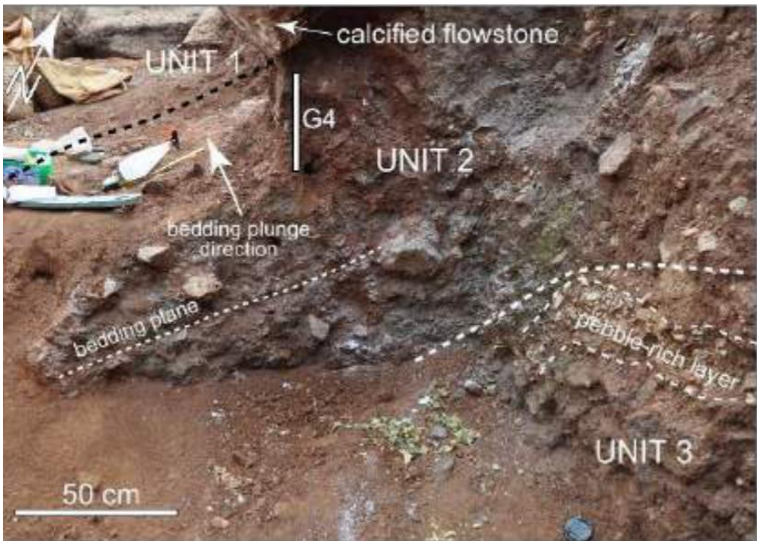

Figure 1. Outcrop at Kromdraai showing the sedimentary units below the calcified flowstone (CF). Note the NNW feature of the basal bedding plane (dashed white line). Three sedimentary units can be distinguished. The pebble-rich layer is located at the base of sedimentary Unit O (unpublished data)

Methodology (short):
The method involves removing a small sample of enamel (~30-50 mg) and then removing all other dental components (e.g. dentine) from the sample. The sample is powdered and bleached before being subsampled for analysis of two separate fractions of amino acids. The samples are analysed by RP-HPLC.

Confirmation/permit by museum: Curator letter

Damage/destructive analysis? (if yes, explain in detail)
Yes, to conduct our planned IcPD analysis, we need to destructively sample small portions of enamel from multiple teeth and from several species. We intend to conduct replicate analysis on each tooth and to achieve this we will require samples that yield enamel masses between 15 - 30 mg. As the tooth chip will contain multiple dental components (enamel, dentine, and/or cementum), and thus the masses of the removed chips need to be greater than 30 mg. For previous analyses, chips of approximately 1 cm^2 have yielded successful analyses, so this is the size of sample we would aim to collect, depending on the thickness of the enamel (Figure 2).

The sampling location can be any part of the tooth, so it is often desirable to take a sample from an area of the tooth that has already fractured or broken off (e.g. Figure 2). This makes the task of sampling easier (as the enamel is often at the surface) and reduces the visual impact of sampling on the specimen.

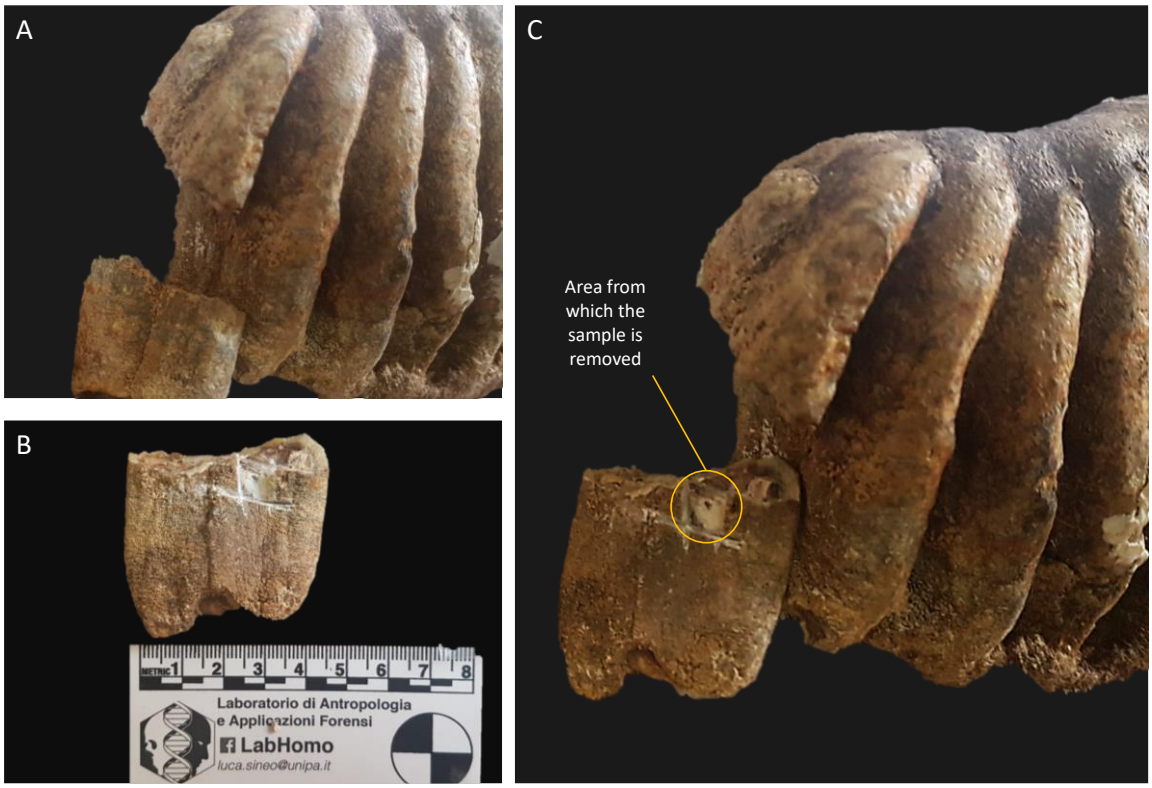

Figure 2. Sampling photos of an elephant tooth. Top left (A) shown the tooth prior to removal of the chip. Bottom left (B) and right (C) show the tooth after sampling. The area of the tooth that has been sampled from is the internal side of the enamel fold and thus if the tooth were to be reattached the sampling location would be hidden. The sampling location is next to a pre-existing fracture where a ~1 cm^2 chip of enamel has been removed.

When choosing a region to sample from for IcPD, it is preferable to take enamel from a region that has undergone the least amount of taphonomic alteration. We have found that significant discolouration of the enamel, usually iron sulphate staining, can alter the IcPD analysis. Therefore, when the option is available, it is preferable to choose a sampling region that visually appears to have undergone less discolouration (Figure 3). Sample chips are removed using a precision drill.

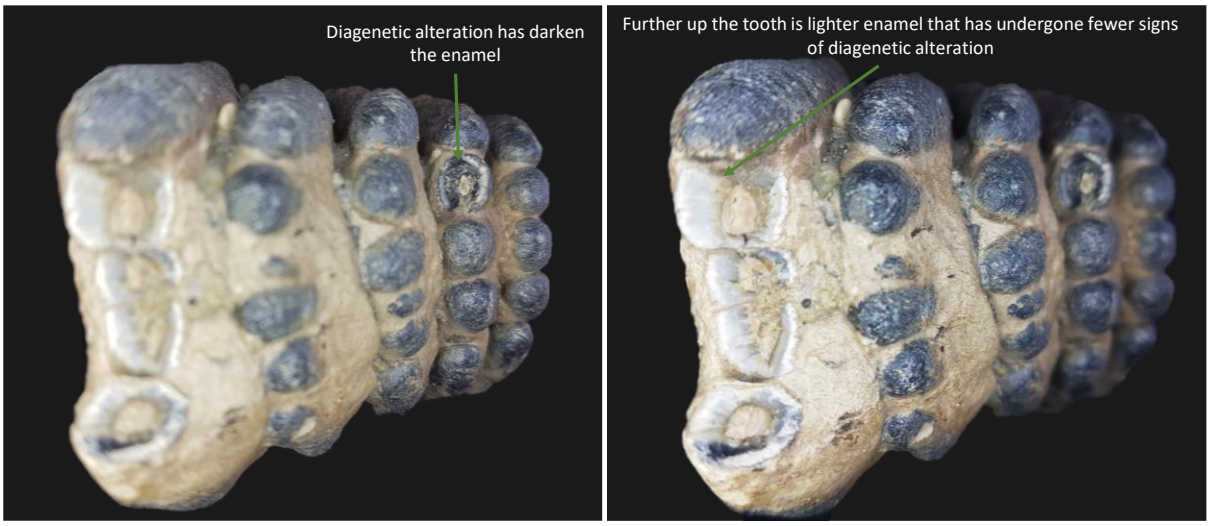

Figure 3. The basal region (roots) of an elephantid tooth. Left: the green arrow indicates an unsuitable area for enamel IcPD sampling, due to visible signs of taphonomic alteration of the mineral, probably caused by the inclusion of iron sulphate. Right: the green arrow indicates a more suitable area for sampling, due to fewer visible signs of alteration.

Once the sampled chips have been cleaned of any additional dental components, the enamel is then powdered with an agate pestle and mortar, and prepared using modified procedures of Penkman et al. (2008), but optimized for enamel (Dickinson et al., 2019).

Statement why this study cannot be done in South Africa:
See previsous