Survival of eggshell peptides over millions of years in Africa is due to mineral binding

Beatrice Demarchi¹, Shaun Hall², Teresa Roncal-Herrero³, Colin L. Freeman², Jos Woolley¹, Molly K. Crisp⁴, Julie Wilson^{4,5}, Anna Fotakis⁶, Roman Fischer⁷, Benedikt Kessler⁷, Rosa Rakownikow Jersie-Christensen⁸, Jesper V. Olsen⁸, James Haile⁹, Jessica Thomas^{6,10}, Curtis W. Marean^{11,12}, John Parkington¹³, Samantha Presslee¹, Julia Lee-Thorp⁹, Peter Ditchfield⁹, Jacqueline F. Hamilton¹⁴, Martyn W. Ward¹⁴, Chunting Michelle Wang¹⁴, Marvin D. Shaw¹⁴, Terry Harrison¹⁵, Manuel Domínguez-Rodrigo¹⁶, Ross D. E. MacPhee¹⁷, Amandus Kwekason¹⁸, Michaela Ecker⁹, Liora Kolska Horwitz¹⁹, Michael Chazan^{20,21}, Roland Kröger³, Jane Thomas-Oates^{4,22}, John H. Harding², Enrico Cappellini⁶, Kirsty Penkman⁴, Matthew J. Collins^{*}

Affiliations

- ¹ BioArCh, Department of Archaeology, University of York, York, YO10 5DD, United Kingdom.
- ² Department of Material Science and Engineering, University of Sheffield, Sheffield, S1 3JD, United Kingdom.
- ³ Department of Physics, University of York, York, YO10 5DD, United Kingdom.
- ⁴ Department of Chemistry, University of York, York, YO10 5DD, United Kingdom.
- ⁵ Department of Mathematics, University of York, York, YO10 5DD, United Kingdom.
- ⁶ Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, Øster Voldgade 5-7, 1350 Copenhagen, Denmark.
- ⁷ Advanced Proteomics Facility, Target Discovery Institute, Nuffield Department of Medicine, University of Oxford, Roosevelt Drive, Oxford OX3 7FZ, United Kingdom.
- ⁸ Novo Nordisk Foundation Center for Protein Research, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen, Denmark.
- ⁹ Research Laboratory for Archaeology and the History of Art, University of Oxford, South Parks Road, Oxford, OX1 3QY, United Kingdom.
- ¹⁰ Molecular Ecology and Fisheries Genetics Laboratory, School of Biological Sciences, Deiniol Road, Bangor University, Bangor LL57 2UW, United Kingdom.
- ¹¹ Institute of Human Origins, SHESC, Arizona State University, 85287-4101, USA.
- ¹² Centre for Coastal Palaeoscience, Nelson Mandela Metropolitan University, Port Elizabeth, Eastern Cape 6031, South Africa.
- ¹³ Department of Archaeology, University of Cape Town, Rondebosch 7701, South Africa.
- ¹⁴ Wolfson Atmospheric Chemistry Laboratories, Department of Chemistry, University of York, York, YO10 5DD, United Kingdom.
- ¹⁵ Center for the Study of Human Origins, Department of Anthropology, New York University, 25 Waverly Place, New York, NY 10003, USA.
- ¹⁶ Department of Prehistory, Complutense University, Ciudad Universitaria s/n, 28040 Madrid, Spain
- ¹⁷ Department of Mammalogy, American Museum of Natural History, New York, NY 10024 USA.

Protein sequences can complement ancient DNA in reconstructing evolution and phylogeny, and extend the reach of biomolecular studies into deep time. It is a universal truth that proteins survive better than DNA, but for how long can they *really* withstand the combined effect of time and temperature? Claims of intact dinosaur protein sequences are in sharp contrast with the extent of degradation consistently found in closed-system biominerals and predicted by kinetic models. Indeed, the very mechanisms for exceptional survival have not yet been clarified.

Here we use an unprecedented combination of rigorous, multi-analytical testing of the authenticity of ancient protein sequences and computational modelling of protein-mineral interactions. We target ostrich eggshell from sites spanning the last 4 million years in Tanzania and South Africa and we unequivocally demonstrate the survival of peptide sequences in paleontological eggshell which has endured the combined effect of temperature and time for the equivalent of ~ 15 million years at 10°C (thermal age). The peptide sequence surviving consistently in the oldest eggshells is chemically unstable but calculations of the binding energy show that it is strongly bound to the mineral surface. The effect of binding is loss of entropy at the mineral surface, lowering the effective temperature of the local environment, so that the peptides are "frozen" to the surface.

Biomineralising proteins from eggshell, shell and other organic-inorganic biomaterials have the best potential for preservation in the fossil record over geological timescales and can therefore help to answer fundamental questions about the past.

¹⁸ National Museum of Tanzania, P.O. Box 511, Dar es Salaam, Tanzania.

¹⁹ National Natural History Collections, Faculty of Life Sciences, The Hebrew University, Berman Building, Edmond Safra Campus, 91904 Jerusalem, Israel.

²⁰ Department of Anthropology, University of Toronto, Toronto, ON M5S 2S2, Canada.

²¹ Evolutionary Studies Institute, University of the Witwatersrand, 1 Jan Smuts Avenue, Braamfontein, Johannesburg 2000, South Africa.

²² Centre of Excellence in Mass Spectrometry, University of York, York, YO10 5DD, United Kingdom.