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In situ detection of bound Ni-tetrapyrrole moieties in ~1 Gyr-old eukaryote microfossil suggesting its phototrophy

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The acquisition of photosynthesis is a fundamental step in the evolution of the eukaryotes, leading to the complexification of trophic networks and making possible the later colonization of land. Few phototrophic organisms are unambiguously recognized in the Proterozoic fossil record and therefore, the timing of the onset of eukaryotic photosynthesis and its evolution in the different groups are still poorly understood. *In situ* detecting metabolic byproducts in individual microfossils is then the key for a direct identification of their metabolisms, but up to now, has remained elusive.

Tetrapyrroles are pivotal constituents of the cell metabolism in all three domains and the main biological tetrapyrroles are chlorophylls and hemes. Whereas tetrapyrroles commonly degrade in the early phases of burial and diagenesis, they may transform into geoporphyrins under favourable conditions. Geoporphyrins are common in bulk solvent extracts of Phanerozoic sedimentary rocks but exceedingly rare in the Precambrian. However, current approaches do not allow association of the detected porphyrins to individual fossils. Such analyses can also not be performed on overmature rocks because even relatively mild thermal alteration around 200°C is incompatible with the preservation of free biomarkers.

Combining morphological, chemical and ultrastructural analyses with synchrotron-based X-ray Fluorescence (SR-XRF) and X-ray Absorption Spectroscopy (SR-XAS), we identify bound nickel-tetrapyrrole moieties in low-grade metamorphic rocks (<200°C), preserved *in situ* within cells of *Arctacellularia tetragonala*, a ~1 Gyr-old multicellular eukaryote from the Congo basin (Mbuji-Mayi Supergroup, Democratic Republic of the Congo). We interpret the tetrapyrrole moieties as chlorophyll derivatives, and *A. tetragonala* as one of the earliest algae, part of the Archaeaplastidae supergroup.

This new methodology, applicable to billion-of-year old, overmature rocks, provides new constraints on the evolution of phototrophy during the Precambrian and the diversification of primary producers in early ecosystems.