SUPPLEMENTARY INFORMATION FOR

Molecular preservation of the pigment melanin in fossil melanosomes

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Supplementary Figure S1 | Single-element EDX maps of the head region and surrounding sediments of FUM-N-2050 (white = high intensity, black = low intensity). Note relatively high levels of carbon (C) and sulphur (S) in the eye. Scale bar: 2 mm.
Supplementary Figure S2 | TEM-image of microbodies removed from the eye of FUM-N-2050. Note that the structures have a more-or-less homogenous internal content. The fracturing apparent in some specimens presumably occurred during the preparation of the sample. The overall appearance of the microbodies is consistent with that of modern retinal melanosomes examined by the same technique (see ref. 37). Scale bar: 0.5 µm.
Supplementary Figure S3 | Negative ion ToF-SIMS spectra from melanin and FUM-N-2050 (the latter recorded at high mass resolution and high image resolution, respectively). Note close agreement between the two melanin standard spectra (one synthetic and one natural) – a strong indication that these samples are compositionally similar to one another. Note also close agreement between the two fossil fish eye spectra, to demonstrate the equivalence in the data obtained by using the two different acquisition modes.
Supplementary Figure S4 | Secondary ion images from ToF-SIMS analyses of FUM-N-2050. Signal intensity images of characteristic ions for melanin (top row), silica (second row from the top) and the tape substrate (third row from the top), where the images on the right hand side show the added signal intensities of each component. The bottom row shows (left) a three-colour overlay image of melanin (red), silica (green) and tape (blue), (centre) a total ion image (mainly reflecting the sample topography) with four marked areas from which the spectra in Supplementary Fig. S5 were acquired, and (right) a semitransparent ion image of the m/z 50 (melanin) signal intensity superimposed onto a SEM image to demonstrate the close spatial correlation between the melanin ion signal and the melanosome-like microbodies in the sample from the fossil fish eye. M: m/z value of the imaged ion peak; mc: maximum ion count per pixel; tc: total count in the entire image.
Supplementary Figure S5 | Secondary ion spectra from ToF-SIMS analyses of FUM-N-2050. Comparison of negative ion spectra acquired from different parts of the surface depicted in Supplementary Fig. S4 (bottom row, centre image) together with a standard melanin spectrum, a spectrum from sediments adjacent to the fish fossil, and a spectrum from the tape substrate. Note close spectrum agreement between melanin and the red and green areas, between the sediment and blue area, and, finally, between the tape and purple area. The total area spectrum shows strong contributions from melanin-specific ions, indicating that a significant part of the analysed area contains this molecule.
Supplementary Figure S6 | Negative ion ToF-SIMS spectra of melanin, fossil microstructures and three modern microbial mats. High mass resolution negative ion spectra of (from top to bottom) a synthetic melanin standard, microbodies from the eye of FUM-N-2050, two modern microbial mats (containing cyanobacteria) from Äspö, and a methanotrophic microbial mat from the Black Sea\textsuperscript{38}.
Supplementary Figure S7 | Negative ion ToF-SIMS spectra of melanin, fossil microstructures and three modern microbial mats. (a,b) Expanded mass regions of the negative ion spectra presented in Supplementary Fig. S6 to demonstrate the close agreement between the fossil microbodies and the melanin standard.
Supplementary Figure S8 | Proposed structure of melanin and two porphyrin-related molecules. (a) hemin chloride, (b) proposed oligomeric structure of melanin and (c) chlorophyll a (structures modified from MP Biomedicals, ref. 36 and Sigma Aldrich, respectively).
Supplementary Figure S9 | Negative ion ToF-SIMS spectra of FUM-N-2050, synthetic melanin, chlorophyll \( a \), and hemin chloride. Note significant deviations in the spectra from chlorophyll \( a \) and hemin chloride in comparison to the fossil fish eye spectrum, whereas the melanin spectrum shows a detailed agreement with the fish eye spectrum throughout the entire mass range. The added quadratic deviation from the fossil fish eye spectrum (calculated from normalised intensities of all peaks in Table 1 at m/z \( \geq 48 \)) was 6.6 and 4.5 times smaller for the melanin spectrum when compared to those for chlorophyll \( a \) and hemin chloride, respectively.
Supplementary Figure S10 | Infrared absorbance spectra of melanin (*Sepia*), hemin chloride, chlorophyll *a*, and microbodies (eye) from FUM-N-2050. Note extreme similarity between the spectra obtained from the fossil fish eye and natural melanin standard, an indication that the degree of preservation of the fossil melanin goes beyond the molecular fragments shown in Figure 3. Note also striking spectral differences between the two porphyrin-related compounds (i.e., hemin chloride and chlorophyll *a*) and the fossil fish eye and melanin standard.
Supplementary Figure S11 | Principal component analysis of infrared absorbance spectra from melanin, two porphyrin-related compounds, and various parts of FUM-N-2050. The signature from the fossil fish eye essentially corresponds to that of the Sepia melanin standard, whereas the three mineralized parts of FUM-N-2050 (i.e., head, body and sediment) are similar to one another but significantly different from the melanin standard. The porphyrin-related compounds (i.e., hemin chloride and chlorophyll a) are also firmly grouped together and clearly different from the various parts of FUM-N-2050. The principal component analysis used Ward’s algorithm, first derivative and vector normalization, and was based on the frequency range 974–3780 cm⁻¹.
Supplementary Figure S12 | Stratigraphical column and surficial exposures of the Fur Formation in Denmark. The position of the +22 to +35 volcanic ash layer interval from which FUM-N-2050 was collected is marked with a red vertical bar. PETM = stratigraphic position of the Paleocene–Eocene thermal maximum. Scale bar: 10 m.
Supplementary Table S1 | Calculated secondary ion yields for selected peaks in the ToF-SIMS spectra from the fossil fish eye and melanin. The similar secondary ion yields indicate a high concentration of melanin in the area from which the fossil fish eye spectrum was acquired.

<table>
<thead>
<tr>
<th>Tentative assignment</th>
<th>Observed mass (m/z)</th>
<th>SI yield FUM-N-2050</th>
<th>SI yield <em>Sepia</em> melanin standard</th>
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<tbody>
<tr>
<td>C₃N</td>
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<tr>
<td>C₆N</td>
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<tr>
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<tr>
<td>C₁₁N</td>
<td>146</td>
<td>0,000330</td>
<td>0,000344</td>
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</table>
Supplementary Methods

Geological setting. Strata belonging to the Fur Formation are currently accessible along coastal cliff sections and in a few inland quarries in north-western Jutland, Denmark, especially on the islands of Fur and Mors (Supplementary Fig. S12). Lithologically, the approximately 60 m thick sedimentary sequence comprises a laminated, siliceous to clayey diatomite of early Eocene (Ypresian) age, interbedded with 179 distinct volcanic ash layers numbered from -39 to +140\textsuperscript{22,23} (Supplementary Fig. S12). These ash layers enable a detailed local stratigraphy, and contribute to precise regional correlations\textsuperscript{23}. The sediments were deposited below the storm wave-base in an offshore marine environment under anoxic or slightly dysoxic bottom conditions\textsuperscript{39}. Local upwelling of nutritious bottom water resulted in periodic blooms of diatoms\textsuperscript{39}. Approximately 45–65 % (by weight) of the sediments comprises diatom frustules, whereas clay minerals (30–45 %) and volcanic dust (about 10 %) make up the reminder\textsuperscript{40}.

The formation is regarded as a Konservat-Lagerstätte because of its exceptionally preserved fossils\textsuperscript{41}; these are particularly abundant in the +22 to +35 volcanic ash layer interval (which forms part of the Silstrup Member), and FUM-N-2050 was collected from a calcareous concretion within this part of the stratigraphical column (Supplementary Fig. S12 – red vertical bar).

Specimen details. FUM-N-2050 was collected on the Isle of Fur and comprises a flattened, yet almost complete (tail fin is missing) argentinoid fish with a distinct ‘eye’. Apart from most vertebral centra (which are preserved as negative moulds), the skeleton is solid. Moreover, the specimen is well preserved and largely articulated, although some
vertebrae and cranial elements have been displaced during the process of decay. A pale, phosphatic (determined via energy-dispersive X-ray microanalysis) halo, loosely defining the original body outline, surrounds large parts of the postcrania. Overall length of the specimen is about 36 mm, whereas the brownish eye pigmentation measures approximately 3 mm in diameter.

**Material examined.** In addition to FUM-N-2050, the following substances and structures were examined in this study:

- Synthetic melanin (Fisher Scientific GTF AB).
- Natural melanin from the cephalopod *Sepia officinalis* (Sigma-Aldrich Sweden AB).
- Isolated retinal melanosomes from the cichlid fish *Haplochromis obliquidens* purified as previously described².
- An eye of a juvenile three-spined stickleback fish (*Gasterosteus aculeatus*) prepared as previously described⁴².
- Hemin chloride (MP Biomedicals, LLC).
- Chlorophyll *a* from *Anacytis nidulans* (Sigma Aldrich, C6144).
- Microbial mat reference samples. These were obtained from (i) a methane seep on the NW Black Sea shelf (one sample)³⁸, and (ii) from rock surfaces in the Tunnel of Äspö (Äspö Hard Rock Laboratory, SE Sweden; two samples from sites located at 2200 and 3440 m from the tunnel entrance, respectively). The Black Sea sample is largely comprised of methanotrophic archaea, whereas the Äspö mats represent phototrophic systems with abundant cyanobacteria. The microbial mats were analyzed as cryosections, as previously described³⁸.
Supplementary References


