Supplement of

Insight into the structure of black coatings of ancient Egyptian mummies by advanced electron magnetic resonance of vanadyl complexes

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## Table S1. Description of samples

| Samples | Object | Provenance | Origin /Dating | Description |
|---------|--------|------------|----------------|-------------|
| Ref 1   | Natural asphalt | C2RMF | Dead Sea, floating blocks (Late Cretaceous) | Black solid |
| Ref 2   | Bitumen of Judea | C2RMF | Commercial | Brown powder |
| Hum 1   | Anthropomorphic coffin | The Art and History museum of Narbonne, France (Ref: C2RMF76267) | Upper Egypt (Abydos ?). Ptolemaic period (332 BC – 30 BC) | Coffin of Irethorerou, servant of Khonsou, of the White Crown and of Horus. Black matter covering the bottom of the coffin |
| Hum 2   | Human mummy | The Hieron museum, Paray-le-Monial, France (Ref: FZ30827) | Late Period, end of the IVth century BC | Mummy of a 35-45 years old man, named …djeb. Set of crossed bands, coated with dark matter |
| Hum 3   | Human mummy | Museum of Boulogne, France (Ref. 35906) | Late period, XXVth dynasty (744 BC - 656 BC) | Mummy found in the coffin of Nehemsimontou, coated with black matter. |
| An 1    | Ram mummy | The Louvre museum, Paris, France; (Ref: C2RMF 64621) | Upper Egypt (Elephantine). Late period (672 BC–322 BC) | Fragment of black matter covering the mummy |
| An 2    | Ram mummy | The Thomas Dobrée museum, Nantes, France (Ref: C2RMF36230) | Upper Egypt. Late Period (664 BC – 332 BC). | Fragments of black matter covering the mummy. |
| An 3    | Ram mummy (the same as An 2) | The Thomas Dobrée museum, Nantes, France (Ref: C2RMF36230) | Upper Egypt. Late Period (664 BC – 332 BC). | Fragments of tissue strips covering the mummy, coated with a brown material |
| An 4    | Crocodile mummy | Musée des confluences, Lyon, France (Ref: 90001841) | Upper Egypt (Kom Ombo). Ptolemaic period | Posterior part of mummified crocodile skull, covered with black matter. |
Figure S1. Binocular photographs of the samples studied in this work. © C2RMF.
S2 EPR spectra:

Figure S2. EPR spectra at X band and at room temperature of bitumen reference and human mummies: (a) *Ref 2*, (b) *Hum 3* and (c) *Hum 2*. This highlights the lack of VO-nP complexes (green circles) in *Ref 2* and *Hum 3*. (From Dutoit, et al., 2020)

Figure S3. EPR spectra at X band and at room temperature of animal mummies, highlighting EPR lines of VO-nP complexes in (green circles). (From Dutoit, et al., 2020)
Figure S4. EPR spectra at Q band and at 100 K of the reference bitumen and samples of black coatings.
**Figure S5.** $^1$H ENDOR spectra at Q band and at 100K of the reference bitumen and samples of black coatings. The field setting values are 1230.9 mT (Ref 1), 1225.4 mT (Ref 2), 1230.6 mT (Hum 3), 1253.2 mT (An 1), 1252.8 mT (An 2), 1228.1 mT (An 3), 1253.1 mT (An 4), 1222.7 mT (Hum 1) and 1252.9 mT (Hum 2).

**Figure S6.** Two examples of geoporphyrins commonly found in oil, with the corresponding parent biomolecules
S4 Derivation of Equation 1

The $^1$H ENDOR spectrum is the superposition of two independent signals: (i) one from the protons of the C-H meso bridges linking pyrole groups of porphyrin ligands, hereafter referred to as VOP-$^1$H, and (ii) the other one from the matrix protons, hereafter referred to as M-$^1$H, corresponding to protons of asphaltene, of the natural substances of the black matter, and of protons of alkyl substituent in porphyrin ligands. M-$^1$H protons are characterized by a pure dipolar hf interaction while VOP-$^1$H protons are characterized by an isotropic hf interaction in addition to the dipolar one.

Let $X$ be the signal height at the frequency $\nu_\parallel$ corresponding to the parallel component of the VOP-$^1$H signal and $Y$ the signal height at the maximum of perpendicular component of the VOP-$^1$H at frequency $\nu_\perp$ (see Fig.4a). Let also $X_{\text{VOP}}, X_M, Y_{\text{VOP}}$ and $Y_M$ be the respective contributions of a single VOP molecule and a single M-$^1$H to $X$ and $Y$.

Then:

$$ \frac{X}{Y} = \frac{N_{\text{VOP}}X_{\text{VOP}} + N_M X_M}{N_{\text{VOP}}Y_{\text{VOP}} + N_M Y_M} $$  \hspace{1cm} \text{(S1)} $$

where $N_{\text{VOP}}$ and $N_M$ are the total numbers of VOP molecules and matrix protons in the sample, respectively.

The VOP molecules are embedded in bitumen aggregates spread within a bioorganic matrix, which contains the M-$^1$H’s. As the M-$^1$H’s are detected upon saturating an EPR transition of the VOP molecules, they must have a residual dipolar hf interaction with the VOP’s. We thus assume that the detected M-$^1$H’s are in a layer of volume $V_L$ surrounding a bitumen aggregate (Fig. S7), then $N_M = N_A V_L [H]$ where $N_A$ is the total number of bitumen aggregate in the sample and $[H]$ the concentration of M-$^1$H’s in the matrix. We also have $N_{\text{VOP}} = [VOP] V$, with $[VOP]$ the concentration of VOP’s in the sample and $V$, the sample volume. As the experimental variable is $x = [VOP]/[VOP]_{\text{ref}}$, where $[VOP]_{\text{ref}}$ is the VOP concentration in the reference sample Ref 1, $N_{\text{VOP}}$ is then rewritten as $N_{\text{VOP}} = x[VOP]_{\text{ref}} V$, yielding: $\frac{N_M}{N_{\text{VOP}}} = a \frac{x}{x}$, with $a = N_A V_L [H] / [VOP]_{\text{ref}}$. Finally, we obtain:

$$ \frac{X}{Y} = \frac{X_{\text{VOP}}}{Y_{\text{VOP}}} \times \frac{x + a X_M / X_{\text{VOP}}}{x + a Y_M / Y_{\text{VOP}}} $$  \hspace{1cm} \text{(S2)} $$

From the ENDOR spectrum of Ref 1 dominantly made of the contribution of VOP-$^1$H signal and negligible contribution from M-$^1$H, we get $\frac{X_{\text{VOP}}}{Y_{\text{VOP}}} \approx 0.625$ and assuming a gaussian lineshape for the M-$^1$H ENDOR line, we get $\frac{X_M}{Y_M} \approx 0.03$ giving $\frac{X_M}{X_{\text{VOP}}} \approx 0.048 \frac{Y_M}{Y_{\text{VOP}}}$ and finally:
\[
\frac{X}{Y} \approx 0.625 \times \frac{x + 0.048 \times b}{x + b}
\]  \hspace{1cm} (S3)

with a single adjustable parameter \( b = a \frac{Y_M}{Y_{VOP}} \), which depends on the sizes and dispersion of the bitumen aggregates through \( N_A \) and the ratio \( \frac{\nu_L}{\nu} \).

**Figure S7.** Schematic description of a bitumen aggregate in interaction with protons of bioorganic compounds.
S5 HYSCORE spectra of Ref 2

Figure S8. HYSCORE spectra of Ref 2 recorded by observing the two EPR transitions $m_I = -1/2$ and $m_I = +3/2$. Figures on the right show the portions of spectra corresponding to the frequency range of dq-dq correlations. Correlations sq-dq are not clearly detected because the VO-P content is lower in Ref 2 than in Ref 1.

S6 Simulation of HYSCORE spectra

Figure S9. Effect of $\tau$ values on simulated dq-dq correlation peaks for VO-P1 (in green) and VO-P2 (in red) complexes, showing the lack of blind spot effects. Field setting $m_I = +3/2$ at 355.6 mT
The first order nuclear spin energy levels of a single \( m_s \) state of VO\(^{2+} \) interacting with the nuclear spin \( I = 1 \) of a \(^{14}\)N nucleus is given by:

\[
E = \pm \frac{1}{2} A m_I + Q \left( \frac{3}{2} m_I - 1 \right) - \nu N m_I
\]  

where the energy \( E \), the hf interaction \( A \) and the quadrupolar interaction \( Q \) are taken along the direction of the magnetic field. The corresponding energy level diagram is given in Fig. S9 for the two \( m_s \) states. The frequencies of the single quantum (\( \Delta m_I = \pm 1 \)) and double quantum (\( \Delta m_I = \pm 2 \)) nuclear spin transitions of \(^{14}\)N are given by (Reijerse, et al., 1998; Dikanov, et al., 2004):

\[
v_{1sq}^\pm = A \pm \nu N + \frac{3Q}{2} + (2^{nd} \text{ order terms})
\]  

\[
v_{2sq}^\pm = A \pm \nu N - \frac{3Q}{2} + (2^{nd} \text{ order terms})
\]  

The second order corrections \( \nu^{(2)} \) to the single quantum frequencies \( v_{1sq}^\pm \) and \( v_{2sq}^\pm \) are:
\[ v_{1,2sq}^{(2)+} = \left( A^{(2)} \pm Q^{(2)} \right) / (A - 2\nu_N) \]
\[ v_{1,2sq}^{(2)-} = \left( A^{(2)} \pm Q^{(2)} \right) / (A + 2\nu_N) \]
where
\[ A^{(2)} = 1/4 \left( T_{np}^2 + T_{nq}^2 \right) + K^2 \left( 3 + \eta^2 \right) - 3\eta^2 / 4 \]
\[ Q^{(2)} = 3 \left( \eta T_{np} + \eta T_{nq} \right) \]

\( K = e^2 qQ / 4h \) is the quadrupolar coupling constant. The matrix elements \( T_{np} \) and \( T_{nq} \) in \( A^{(2)} \) and \( Q^{(2)} \) are anisotropic components of the hf interaction, \( n \) is the orientation of the magnetic field, and \( p \) and \( q \) are two orientations perpendicular to \( n \) and to each other.

Determination of \( A \) from expressions of \( V_{dq}^\pm \) is not affected by \( 2^{nd} \) order correction:

\[ A = \frac{2\nu_N \left( v_{dq}^+ + v_{dq}^- \right)}{8\nu_N - v_{dq}^+ - v_{dq}^-} \]  

On the contrary, measurement of \( Q \) from expressions from Eqs.S5 is affected by second order corrections and necessitates the preliminary determination of \( V_{1sq}^\pm \) and \( V_{2sq}^\pm \). As only a part of the sq-dq correlations has been detected, only half of them sq frequencies could be determined precisely. The observed sq-dq correlations for VO-P1 and VO-P2 correlate the dq transition of the \( m_s = +1/2 \) state with one of the sq transitions of the \( m_s = -1/2 \) state, and recalling that \( v_{1sq}^+ + v_{2sq}^+ = V_{dq}^+ \), all transitions in the \( m_s = -1/2 \) state are known without uncertainty due to \( 2^{nd} \) order corrections. Single-quantum transitions in the \( m_s = +1/2 \) state were obtained to \( 1^{st} \) order by the equation
\[ v_{1sq}^+ - v_{1sq}^- \approx v_{2sq}^+ - v_{2sq}^- \approx 2\nu_N \] and are thus affected by \( 2^{nd} \) order corrections. The resulting diagrams for VO-
P complexes are given in Fig.S9. In the absence of unambiguous sq-dq correlations for VO-P3 and VO-P4, we could not obtain sq frequencies and quadrupolar parameter \( Q \) for these complexes.

The second order term in Eqs. S5, S6, S7 and S8 can be estimated as follows. Combining the two dq frequencies gives:

\[ v_{dq}^+ - v_{dq}^- = 4\nu_N - \frac{2\nu_N A^{(2)}}{A^2 / 4 - \nu_N^2} \]

From the experimental values of \( v_{dq}^\pm \) and from \( \nu_N = 1.1 \) MHz, we obtain \( A^{(2)} = 0.55 \) MHz and 0.44 MHz in VO-P1 and VO-P2, respectively. This gives a second order contribution \( \frac{A^{(2)}}{(A/2)^\pm \nu_N} \approx 0.1 - 0.2 \) MHz in Eq. S6 for VO-P complexes, which corresponds also to the uncertainty in the experimental measurement of dq frequencies in Fig. 6.

Concerning second order contributions in the determination of the quadrupolar interaction \( Q \), expressions for sq frequencies give
\[ v_{1sq}^+ - v_{2sq}^+ = 3Q + \frac{Q^{(2)}}{A/2 + \nu_N} \] and \[ v_{1sq}^- - v_{2sq}^- = 3Q + \frac{Q^{(2)}}{A/2 - \nu_N} \].
which gives an estimation $Q^{(2)} \approx 0.5$ MHz of the same order as $A^{(2)}$, and thus $\frac{Q^{(2)}}{A/2 \pm \nu_N} \approx 0.1 - 0.2$ MHz.

| VO-P1 | VO-P2 | VO-P4 | VO-P3 |
|-------|-------|-------|-------|
| $a_{\text{iso}} = 7.3$ MHz | $a_{\text{iso}} = 6.5$ MHz | $a_{\text{iso}} = 6.8$ MHz | $a_{\text{iso}} \approx (7.3$ MHz) |
| $|Q| = 0.50 \pm 0.03$ MHz | $|Q| = 0.55 \pm 0.02$ MHz |

\[
\begin{array}{c c c c}
\hline
m_I &=+1/2 & & \\
&\hline
V_{1\text{sq}} & V_{\text{dq}} & 5.5 & 9.5$ MHz \\
V_{2\text{sq}} & & 4.0 & \\
\hline
& & & \\
\end{array}
\begin{array}{c c c c}
\hline
m_I =-1/2 & & & \\
&\hline
V_{1\text{sq}} & V_{\text{dq}} & 3.3 & 5.3$ MHz \\
V_{2\text{sq}} & & 2.0 & \\
\hline
& & & \\
\end{array}
\begin{array}{c c c c}
\hline
& & & \\
V_{1\text{sq}} & V_{\text{dq}} & 5.3 & 8.9$ MHz \\
& & 3.6 & \\
\hline
& & & \\
\end{array}
\begin{array}{c c c c}
\hline
& & & \\
\hline
& & & \\
\end{array}
\]

\[
\begin{array}{c c c c}
\hline
m_I &=+1/2 & & \\
&\hline
V_{1\text{sq}} & V_{\text{dq}}^+ & 5.3 & 8.9$ MHz \\
V_{2\text{sq}} & & 4.0 & \\
\hline
& & & \\
\end{array}
\begin{array}{c c c c}
\hline
m_I =-1/2 & & & \\
&\hline
V_{1\text{sq}} & V_{\text{dq}}^- & 3.1 & 4.6$ MHz \\
V_{2\text{sq}} & & 2.0 & \\
\hline
& & & \\
\end{array}
\begin{array}{c c c c}
\hline
& & & \\
V_{1\text{sq}} & V_{\text{dq}} & 5.7 & 9.7$ MHz \\
& & 3.6 & \\
\hline
& & & \\
\end{array}
\begin{array}{c c c c}
\hline
& & & \\
V_{1\text{sq}} & V_{\text{dq}} & 5.7 & 10$ MHz \\
& & 3.1 & \\
\hline
& & & \\
\end{array}
\]

**Figure S11.** Energy level diagram of an electron spin $S = 1/2$ interacting with a nuclear spin $I = 1$, showing single quantum (sq) transitions (in black) and double quantum (dq) transitions (in red), with the corresponding diagrams for the four VO-Ps detected in the black matter; the four experimental diagrams correspond to the observation of the EPR transition $m_I = +3/2$. (for VO-P1, VO-P2 and VO-P4) and $m_I = -1/2$ for VO-P3; the quadrupolar interaction can be measured from sq transitions only when sq-dq peaks are detectable (VO-P1 and VO-P2); $a_{\text{iso}}$ was deduced from dq-dq transitions obtained with the two EPR transitions $m_I = -1/2$ and $m_I = +3/2$. 

| VO-P1 | VO-P2 | VO-P4 | VO-P3 |
|-------|-------|-------|-------|
| $a_{\text{iso}} = 7.3$ MHz | $a_{\text{iso}} = 6.5$ MHz | $a_{\text{iso}} = 6.8$ MHz | $a_{\text{iso}} \approx (7.3$ MHz) |

| $|Q| = 0.50 \pm 0.03$ MHz | $|Q| = 0.55 \pm 0.02$ MHz |