



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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27 S1 Samples

29 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 ?). Ptolemaïc 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 IV th century BC	Mummy of a 35-45 years old man, nameddjeb. Set of crossed bands, coated with dark matter
Hum 3	Human mummy	Museum of Boulogne, France (Ref. 35906)	Late period, XXV th 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). Ptolemaïc period	Posterior part of mummified crocodile skull, covered with black matter.





Figure S1. Binocular photographs of the samples studied in this work. © C2RMF.



42 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 S5. ¹H ENDOR spectra at Q band and at 100K of the reference bitumen and samples of black coatings. The field setting values are 1230.9 (*Ref 1*), 1225.4 mT (*Ref 2*), 1230.6 mT (*Hum 3*), 1253.2 mT (*An 1*), 1252.8 mT (*An 2*), 1228.1 (*An 3*), 1253.1 mT (*An 4*), 1222.7 mT (*Hum 1*) and 1252.9 mT (*Hum 2*).

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Figure S6. Two examples of geoporphyrins commonly found in oil, with the corresponding parent biomolecules

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S4 Derivation of Equation 1

62 The ¹H 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, hereafeter referred to as VOP-¹H, and (ii) the other one from the

64 matrix protons, hereafeter referred to as M-¹H, corresponding to protons of asphaltene, of the natural substances of

the black matter, and of protons of alkyl substituent in porphyrin ligands. M-¹H protons are characterized by a pure
 dipolar hf interaction while VOP-¹H protons are characterized by an isotropic hf interaction in addition to the dipolar

60 alpoial in interaction while vor - it protons are characterized by an isotropic in interaction in addition to the dipolar67 one.

68 Let *X* be the signal height at the frequency v_{\parallel} corresponding to the parallel component of the VOP-¹H signal and *Y*

69 the signal height at the maximum of perpendicular component of the VOP-¹H at frequency ν_{\perp} (see Fig.4a). Let also

- 70 X_{VOP}, X_M, Y_{VOP} and Y_M be the respective contributions of a *single* VOP molecule and a *single* M-¹H to X and Y.
- 71 Then :

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

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

74 The VOP molecules are embedded in bitumen aggregates spread within a bioorganic matrix, which contains the M-

¹H's. As the M-¹H'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-¹H'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-¹H's in the matrix. We also have $N_{VOP} = [VOP]V$, with [VOP] the

concentration of VOP's in the sample and V, the sample volume. As the experimental variable is x =

80 $[VOP]/[VOP]_{ref}$, where $[VOP]_{ref}$ is the VOP concentration in the reference sample **Ref 1**, N_{VOP} is then rewritten

81 as
$$N_{VOP} = x[VOP]_{ref}V$$
, yielding: $\frac{N_M}{N_{VOP}} = \frac{a}{x}$, with $a = N_A \frac{V_L}{V} \frac{[H]}{[VOP]_{ref}}$. Finally, we obtain:

82

$$\frac{X}{Y} = \frac{X_{VOP}}{Y_{VOP}} \times \frac{x + aX_M / X_{VOP}}{x + aY_M / Y_{VOP}}$$
(S2)

83 From the ENDOR spectrum of *Ref 1* dominantly made of the contribution of VOP-¹H signal and negligible

84 contribution from M-¹H, we get $\frac{X_{VOP}}{Y_{VOP}} \approx 0.625$ and assuming a gaussian lineshape for the M-¹H ENDOR line, we get

85 $\frac{X_M}{Y_M} \approx 0.03$ giving $\frac{X_M}{X_{VOP}} \approx 0.048 \frac{Y_M}{Y_{VOP}}$ and finally:

86
$$\frac{X}{Y} \approx 0.625 \times \frac{x + 0.048 \times b}{x + b}$$
(S3)

87 with a single adjustable parameter $b = a \frac{Y_M}{Y_{VOP}}$, which depends on the sizes and dispersion of the bitumen aggregates 88 through N_A and the ratio $\frac{V_L}{V}$.



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Figure S7. Schematic description of a bitumen aggregate in interaction with protons of bioorganic compounds.



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Figure S8. HYSCORE spectra of *Ref 2* recorded by observing the two EPR transitions $m_I = -1/2$ and $m_I = +3/2\perp$. 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*.

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96 S6 Simulation of HYSCORE spectra



Figure S9. Effect of τ 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 $m_I = +3/2 \pm at 355.6$ mT



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Figure S10. Comparison of experimental and simulated HYSCORE spectra of VO-P1 and VO-P2 of sample Ref 1. The experimental spectrum is in grey, the simulated spectra are in green (VO-P1) and red (VO-P2). Field setting $m_I = +3/2 \pm$ at 355.6 mT. The simulation parameters are given in Table 3

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S7 Estimation of second order contributions in ¹⁴N parameters from dq-dq and sq-dq correlation peaks

104 The first order nuclear spin energy levels of a single m_s state of VO²⁺ interacting with the nuclear spin I = 1 of a ¹⁴N nucleus is given by:

106
$$E = \pm \frac{1}{2} A m_I + Q \left(\frac{3}{2} m_I^2 - 1 \right) - v_N m_I$$
 (S4)

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 ¹⁴N are given by (Reijerse, et al.,

110 1998; Dikanov, et al., 2004):

$$v_{1sq}^{\pm} = \frac{A}{2} \pm v_N + \frac{3Q}{2} + (2^{\text{nd}} \text{ order terms})$$

111

112

$$v_{2sq}^{\pm} = \frac{A}{2} \pm v_N - \frac{3Q}{2} + (2^{\text{nd}} \text{ order terms})$$

$$v_{dq}^{\pm} = A \pm 2v_N + \frac{A^{(2)}}{(A/2) \pm v_N}$$
(S5)
(S5)

113 The second order corrections $v^{(2)}$ to the single quantum frequencies v_{1sq}^{\pm} and v_{2sq}^{\pm} are:

114
$$v_{1,2sq}^{(2)+} = \left(A^{(2)} \pm Q^{(2)}\right) / (A - 2\nu_N)$$

$$v_{1,2sq}^{(2)-} = \left(A^{(2)} \pm Q^{(2)}\right) / (A + 2\nu_N)$$
(S7)

115 where

116

$$A^{(2)} = 1/4 \left(T_{np}^{2} + T_{nq}^{2} \right) + K^{2} \left(3 + \eta^{2} \right) - 3Q_{n}^{2} / 4$$

$$Q^{(2)} = 3 \left(Q_{np} T_{np} + Q_{nq} T_{nq} \right)$$
(S8)

 $K = e^2 q Q / 4h$ is the quadrupolar coupling constant. The matrix elements T_{np} and T_{nq} in $A^{(2)}$ and $Q^{(2)}$ are 117 anisotropic components of the hf interaction, **n** is the orientation of the magnetic field, and **p** and **q** are two orientations 118 perpendicular to **n** and to each other. 119

Determination of A from expressions of v_{dq}^{\pm} is not affected by 2nd order correction: 120

121
$$A = \frac{2v_N \left(v_{dq}^+ + v_{dq}^- \right)}{8v_N - \left(v_{dq}^+ - v_{dq}^- \right)}$$
(S9)

On the contrary, measurement of Q from expressions from Eqs.S5 is affected by second order corrections and 122 necessitates the preliminary determination of v_{1sq}^{\pm} and v_{2sq}^{\pm} . As only a part of the sq-dq correlations has been 123 detected, only half of then sq frequencies could be determined precisely. The observed sq-dq correlations for VO-P1 124 125 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 2nd 126 order corrections. Single-quantum transitions in the $m_s = +1/2$ state were obtained to 1st order by the equation 127 $v_{1sq}^+ - v_{1sq}^- \approx v_{2sq}^+ - v_{2sq}^- \approx 2v_N$ and are thus affected by 2nd order corrections. The resulting diagrams for VO-128 129 P complexes are given in Fig.S9. In the absence of unambiguous sq-dq correlations for VO-P3 and VO-P4, we could 130 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: 131

132
$$v_{dq}^{+} - v_{dq}^{-} = 4v_N - \frac{2v_n A^{(2)}}{A^2 / 4 - v_N^2}$$
 (S10)

From the experimental values of v_{dq}^{\pm} and from $v_N = 1.1$ MHz, we obtain A⁽²⁾ = 0.55 MHz and 0.44 MHz in VO-P1 133

- 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 correspondent to the second seco 134
- complexes, which corresponds also to the uncertainty in the experimental measurement of dq frequencies in Fig. 6. 135
- Concerning second order contributions in the determination of the quadrupolar interaction Q, expressions for sq 136

137 frequencies give
$$v_{1sq}^+ - v_{2sq}^+ = 3Q + \frac{Q^{(2)}}{A/2 + v_N}$$
 and $v_{1sq}^- - v_{2sq}^- = 3Q + \frac{Q^{(2)}}{A/2 - v_N}$,

138 which gives an estimation $Q^{(2)} \approx 0.5$ MHz of the same order as $A^{(2)}$, and thus $\frac{Q^{(2)}}{A/2 \pm v_N} \approx 0.1 - 0.2$ MHz.



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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 $m_I = +3/2\bot$ (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_{iso} was deduced from dq-dq transitions obtained with the two EPR transitions $m_I = -1/2$ and $m_I = +3/2\bot$.