Point-by-point reply to referee # 2 for the manuscript:

Insight into the structure of black coatings of ancient Egyptian mummies by advanced Electron magnetic resonance of vanadyl complexes, by CE. Dutoit, L. Binet, H. Fujii, A. Lattuati-Derieux and D. Gourier.

This work presents an unusual application of EPR spectroscopy and related hyperfine techniques to the study of ancient egyptian mummies. The Authors apply ENDOR and HYSCORE spectroscopies to characterize the local environment of vanadyl porphirin complexes contained in the bitumen present in the embalming mixture. Based on different 14N hyperfine couplings, different types of vanadyl porphyrin complexes are identified, which appear to be related to the origin of the mummy. The work is well written and the experiments carefully performed and I think they represent a new and original application of hyperfine techniques. I have a few comments that should be addressed before publication.

Thank you very much for this very positive appraisal of the work

Comment #1: *The different VO species are assigned based on small differences in the dq-dq correlation peaks shown in Figure 6. However, the spectra were recorded using a single tau value (tau=200 ns), which raises some doubts on the effect of blind spots. At least another tau value, possibly with a shorter length (96-100 ns) should be used to firmly assign the dq-dq cross peaks to different VO species and not, for example to the result of a distribution of hf values (strain). A simulation analysis showing the effect of different tau values on the dq-dq cross-peaks assuming the presence of the four VO different species or the effect of strain may be used as a possible alternative to substantiate the Authors assignment.*

Response: As the vanadyl porphyrin content of these natural and historical samples is low (10^{17} to 10^{18} V^{4+} per gram of black matter), the number of spins in the EPR tube was of the order of 10^{15} to 10^{16}, which gives a low signal-to-noise ratio even in cw-EPR (see lines 18-20 of the revised manuscript, in red). Moreover, as a narrow region of the EPR spectrum is excited, the number of spins actually observed is extremely small. For these reasons it took us at least 20 hours to record each HYSCORE spectrum with an acceptable signal-to-noise ratio. It was therefore difficult to record spectra at different tau values. The value of 200ns was chosen from the 3P-ESEEM versus tau and 4P-ESEEM versus tau experiments on Dead Sea asphalt (Ref 1). The value of 200 ns was optimal for observing all the transitions. We repeated the same experiments for the mummy of unknown origin (Hum3) and this value was also canonical. For this sample we pushed a variation of tau up to 600ns and a value of 300ns was also good.

We have simulated the effect of tau on dq-dq spots as suggested by the reviewer (thanks for the suggestion). We simulated the HYSCORE spectra at tau = 100, 150, 200, 250, 300, 350 ns taking into account both VO-P1 and VO-P2 species. The resulting spectra in the dq-dq region are shown below and are reported also in the Supplementary Information Fig.S9 of the revised manuscript. It can be seen that the dq-dq of the two species remain well separated and well delimited at all values of tau.
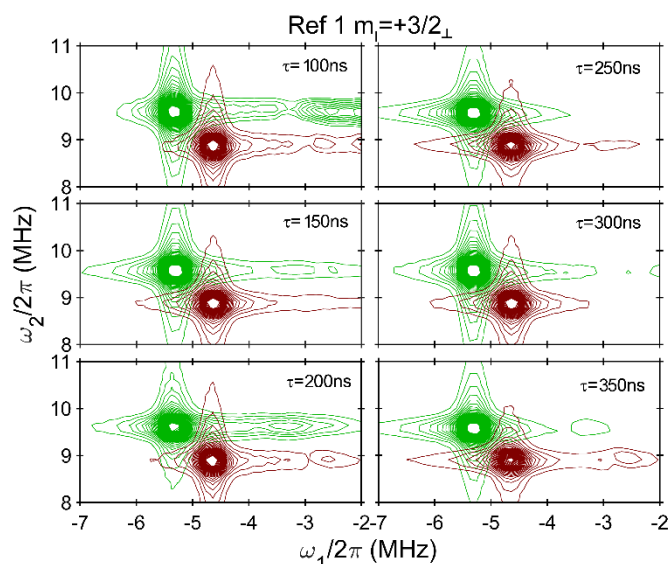
Effect of τ values on simulated dq-dq correlation peaks for VO-P1 and VO-P2 species, showing the lack of blind spot effects.

Comment #2: *It would be interesting to compare the data from these unusual samples with those of related vanadyl phthalocyanine and porphyrin molecular complexes (see for example H. Moons, Z. Phys. Chem. 2017, 231, 887; K. Fukui, J. Phys. Chem. 1993, 97, 11858 and reference therein). In these systems the full 14N hyperfine and nuclear quadrupole tensors are derived. These values may be used as starting point for the simulation of the HYSCORE experiments.*

Response: Thank you for the suggestion. The paper by Moons et al. (2017) has been added in the references. The values measured "manually" (a_{iso} =-7.3 MHz and Q_{zz} = 0.94 MHz) and simulated (a_{iso} = -7.3 MHz and Q_{zz} = 1.0 MHz) for VO-P1 species in our samples are very close to the values a_{iso} = -7.3 MHz and - 7.2 MHz for vanadyl tetraphenyl porphyrin (VOTPP) and vanadyl octaethyl porphyrin (VOEP) in toluene, respectively, with the corresponding values Q of 0.95 MHz and 1.00 MHz, respectively, for the quadrupolar interaction (measured by ESEEM by Fukui et al. 1993). The smaller a_{iso} values measured for VO-P2 (-6.5 MHz) and for VO-P4 (-6.8 MHz) do not seem to have equivalent in the literature, however they are relatively close to those measured by Moons et al. (2017) in vanadyl perfluorophtalocyanine (-6.9 MHz). As phthalocyanines are synthetic molecules, we do not know whether they exist, even in very small quantities, in natural oils and bitumens (see lines 383-387 in the revised manuscript, in red).

Comment #3: *It would be useful to show at least as supplementary material, the simulation of the HYSCORE experiments taken at the two magnetic field settings superimposed to the experimental data. This allows to better judge on the quality of the simulation.*

Response: The superposition of an experimental spectrum and the simulated spectra at tau = 200 ns of VO-P1 and VO-P2 species is shown below and in Figure S10 for the Field setting m_I = +3/2⊥ at 355.6 mT. Most of the features are reproduced. Concerning the field setting m_I = -1/2 at 341.6 mT, this EPR transition suffers from a small, but not zero angular variation. However the excitation bandwidth of the HYSCORE spectrum being smaller than the EPR line width, it is not known which orientation range is selected by the first pulse. For this reason the comparison of the experimental and simulated spectra seems less relevant.
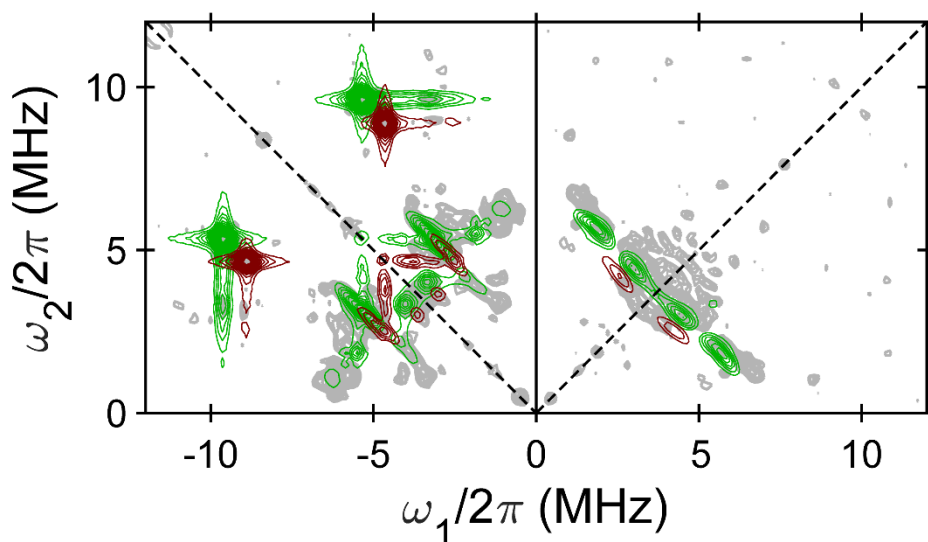
Comment #4: *The negative g-shift (g=1.9994) of the signal assigned to carbon organic radicals of asphaltene should be commented. Do the Authors have a structure in mind for these radical species?*

Response: Thank you for this comment, because the value reported in the manuscript is wrong. We have measured the g-factors on these samples as well as very recently on 14 other samples from a new corpus of mummies. The vast majority of values are around 2.0037, with a few samples deviating slightly from these values, 2.0032 for the lowest, and 2.0042 for the highest. The value 2.0037 (± 2x10^{-4}) is reported in the revised version of the manuscript. Such g-values are linked to the presence of heteroatoms (O, S) in the organic matter (Montanari et al. Appl. Magn. Reson. 14, 81, 1998).

Despite a very large number of EPR analyses of radicals in fossil organic matter (kerogen, coal, asphaltenes etc), there are currently no robust structural models for these radicals. They are characterized above all by their exceptional stability since they are also found in the organic matter of carbonaceous chondrites, the oldest objects in the solar system (~3.5 Ga) (Binet et al. 2002). These could be highly delocalised π-systems based on a phenalenyl-type structures, which are non-kekule structures with an odd number of carbons (Zhang et al. Energy & Fuels, 34, 9094, 2020). However their structure is unknown. The difficulty in proposing a precise structure is probably due to the presence of a distribution of a large number of radicals differing in size, structure and H/C ratio. This issue is challenging for advanced EPR techniques.

Minor points

1. In Table 2 it would be useful to include the assignment to the different VO-P complexes

It was not possible to attribute the EPR parameters in Table 2 to individual VO-Ps, as these natural bituminous materials contain a large number of different vanadyl porphyrins. The resulting EPR spectra are simply the sum of the individual spectra.

2. The value of the magnetic field setting at which the HYSCORE and ENDOR spectra have been recorded should be given in the Figures caption

The values of the magnetic field settings used in ENDOR and HYSCORE experiments are now given in captions of Figs. 4 and 5.