Specific comments

Materials and Methods

- Line 177: *A phase cycle of eight steps*.
  More than one 8-step phase cycling scheme has been proposed for 4-pulse HYSCORE, see *e.g.* DOI 10.1016/0022-2364(90)90181-8 and DOI 10.1007/s00723-008-0140-6.
  It would be beneficial to highlight which one was used and add the corresponding reference.
- Lines 183-184: *The pulse lengths were 1 µs for the first variable mw frequency (mw_2) […]*.
  - What was the amplitude of the HTA pulse ω_{HTA} measured at the observer frequency (mw_1)?
  - Was such a short HTA pulse used indeed?
- Line 207: which specific reference was used for the listed $\boldsymbol{g}$ principal values?

ELDOR-detected NMR

- Line 261: the text mentions the variation of the *spin echo* intensity, however FID-detected EDNMR has been used for this work.
- Lines 261-262: *is detected by a decrement on the spin echo generated by the detection sequence at a variable frequency when it hits an allowed EPR transition*.
  - It may be better to rewrite this sentence as it seems as if the observer frequency mw_1 were swept during the experiment.
  - Also line 263: *are obtained when plotting the echo intensity as a function of the detection frequency (ELDOR frequency)*; this is not in agreement with what is stated in 2.2.3, *first pulse with variable frequency (mw_2) […] second pulse with fixed mw frequency mw_1*.
- Line 269: *Q-band ELDOR-detected NMR is especially suitable for detecting nuclear frequencies of $^{13}C(4a)$*.
  - Some clarification of why this is the case would be beneficial, especially considering the resolution limitations of EDNMR compared to ENDOR.
  - Related to this point, according to the cited references for the spectrometer (Gromov *et al.*, 2001) and the microwave resonator (Tschaggelar *et al.*, 2009) the used equipment should be capable of performing ENDOR measurements. Was there a specific reason to choose EDNMR over Davies ENDOR? Was Davies ENDOR attempted on the studied system?
- Figure 3:
  - The EDNMR spectra are strongly asymmetric. This may impact the quality of the subtraction. How was the experiment set up? Where was the detection frequency placed within the resonator mode?
  - Panel a: the signals at 11 MHz appear in the spectra of both [^{13}C(2)-FMN]-Fld and [^{13}C(2,4a)-FMN]-Fld.
    Is the presence of a signal at 11 MHz in the subtraction spectrum an artefact caused by different acquisition conditions (*e.g.*, different ω_{HTA} field strength, resonator bandwidth, position of the pulses in the resonator mode) between the two experiments?
  - Related to the comment about the *Materials and Methods* section, is the width of the central blind spot compatible with a 1 µs HTA pulse?

    - The *x*-axis of both panels reads *ELDOR frequency (MHz)*.
      This nomenclature may be misleading: the spectra are displayed against the frequency difference between the HTA pulse and the detection frequency; the frequency of the ELDOR source should be in the ~34 GHz range.
    - Line 283 (legend): *which yields no orientation selection*. The figure inset shows indeed some orientation selection; it may be better to use a milder statement, *e.g.* "negligible" or "weak".
    - Line 285 (legend): how were the orientation selection spheres obtained? Which spin Hamiltonian was used? Which pulse excitation bandwidth?
- Line 289: *$2\nu_L(^{13}C) = 13.1$ MHz*. *$\nu_L(^{13}C)$* at the chosen magnetic field should be ~13.11 MHz, hence $2\nu_L(^{13}C)$ is ~26 MHz.
- Line 294: *The difference between the frequency of the detected peaks is close to $2\nu_L(^{13}C)$, which confirms the assignment of the peaks [...]*.
  The frequency difference between the peaks, located at ±30 MHz and ±11 MHz, is approximately 19 MHz.
  This value is rather far from $2\nu_L(^{13}C)$ = 26 MHz.
- Lines 295-297: I would consider rewriting the sentence as, in its current form, it may lack some clarity.
- Lines 300-301: it's not entirely clear to me how the edge of the outermost signal in the EDNMR spectrum could be converted directly into a value of $|A_z|$, especially considering that the system is in the strong-coupling case. Was equation 6, $\nu_+ + \nu_- = |A_z|$, used to estimate $|A_z|$?
- Line 302: I agree with the observation, especially for the outermost signal.
  Were measurements performed at several ω_{HTA} field strengths and/or different lengths of the HTA pulse to ensure the absence of additional broadening due to the choice of the experimental conditions?
- Line 305: is the notion *larger nuclear frequency* related to ν_+?

<u>^{13}C HYSCORE</u>

- The full 2D spectra (both (++) and (-+) quadrants) for [^{13}C(2,4a)-FMN]-Fld are displayed neither in the main text nor in the SI.
    - I find this quite surprising, especially considering that the hyperfine interaction with ^{13}C(4a) is expected to be in the strong coupling regime and should hence give signals in the (-+) quadrant as well.
    - Is the (-+) region of the mentioned spectrum devoid of any signals?
- Line 319: *Since the focus here is on the ^{13}C signals, Fig. 4 only shows the positive quadrant of the 2D measurements*; see the comment above.
- As $I(^{13}C) = ½$, it should be possible to obtain an initial estimate of a_{iso} and |T| by squaring both frequency axes, see DOI 10.1006/jmra.1995.1199.
- Line 334: $|A_3[^{13}C(4a)]|$ is reported to be negative. Is the absolute value a typo?
- Line 334: how were the uncertainties estimated?
- Line 339 (and several other places throughout the manuscript): it would be better to have specific references to figures in the Supplementary Material.
- Figure S.1: it would be better to remind the readers of the field position at which the experiment was performed.

- Figure S.2: it would be better to display the precise location with respect to the EDFS-EPR spectrum at which the experiment was performed. Is it *e.g.* the same as the one in Fig. 3a or a different one?

<u>^{15}N and ^{14}N HYSCORE</u>

- Figures 5 and 6
    - Is there a specific reason for performing the field-edge ^{15}N and ^{14}N experiments on the high-field side of the spectrum rather than on the low-field side? This latter position was *e.g.* chosen for the EDNMR experiments (see Fig. 3a) and for the field-edge ^{13}C-HYSCORE measurement (see Fig. S2).
    - There is some conflict between the main text and the SI: according to the legend of Fig. S5, the experiment would have been performed at the low-field edge of the EPR spectrum whereas according to the corresponding Fig. 6a the measurements were performed on the high-field edge.
    - The field settings are rather different between Fig. 5a (^{15}N) and Fig. 6a (^{14}N). According to which criterion were the edge positions selected?
    - In the legends of Figures 5 and 6 it is mentioned that HYSCORE spectra were recorded at several τ values and summed. How was this performed exactly? Were there major echo intensity changes between the shortest and the longest τ values?
    - Figure 5: at least 4 dash-dot antidiagonal lines are reported. Which nuclei do these correspond to? Considering that the focus is on ^{15}N, it may be better to highlight the corresponding Larmor frequency.
    - Figures 6, S.5 and S.6: why are antidiagonal lines at the ^{2}H Larmor frequency reported? Were the samples partially deuterated?
- Lines 383-384: how were the uncertainties estimated?
  How could some rhombicity of the ^{15}N hyperfine coupling tensors be completely ruled out from the analysis of the experimental data?

<u>General remarks</u>

- I couldn't find any information concerning the availability of the raw data.
- The limited resolution of the figures makes it sometimes challenging to observe the details described and discussed in the text.

Technical corrections

- Line 98: **though* → *through*?
- Lines 133-134: *These results provide with a suitable protocol to experimentally access these coupling and an estimation of the spin density on the Fld model*. This sentence is not entirely clear to me.
- Line 146: **at al.* → *et al.*
- Line 168: although clear to a readership with magnetic resonance background, it may be better to expand the abbreviation *mw* the first time it is used.
- Line 203, equation (1): the hyperfine term does not carry the sum symbol (Σ_i).
- Line 205: **semiquinome* → *semiquinone*.
- Line 212: **use to be* (subject/verb agreement).
- Line 218: **isoaloxacine*; here, as well as in a few other places, the term *isoalloxazine* is spelt incorrectly.
- Line 228: ***4a*** instead of ****4***.
- Line 240, equation (3): the form is not consistent with equation (2) ($A_{||}(^{14}N5)$ in equation 2 vs. $A_z(^{14}N5)$ in equation 3).
- Line 245, equation (4): for the sake of better readability, it would be better to enclose values and the related uncertainties within brackets, *i.e.* (8.4 ± 0.3 mT) - (7.1 ± 0.3 mT).
- Line 261: **on the* → *of the*.
- Figure 3, text between panels *a* and *b*: **Substraction* → Subtraction.
- Line 290: **regimen* → regime.
- Line 294: ^{13}C4a → ^{13}C(4a).
- Line 307: **$A_{x,y}{}^{13}C(4a)]$* → $A_{x,y}[^{13}C(4a)]$.
- Line 315: **carried out in* → carried out on.
- Line 367: **performed in a* → performed on a.
- Line 372: *symmetrical with respect to the diagonal* or antidiagonal?
- Line 376: **HSYCORE* → HYSCORE.
- Line 416: **in Q-band* → at Q-band.
- Line 416: **have* → has.
- Line 423: ** both, hyperfine* → both hyperfine.
- Line 427: **fld* → Fld.
- Line 432: for the sake of completeness, it may be better to stress *Q-band ELDOR-detected NMR*.
- Line 435: **fld* → Fld.
- Line 466: **specific of type* → specific of the type.
- Line 467: **of the of the* → of the.