REFEREE #1

The authors have adressed most points and improved the manuscript, yet some things still need to be clarified.

Orientation Selection:

• No simulation is provided in Figure1 as stated by the authors
In Figure 1, the orientation of the g frame is shown but no simulation is intended. This orientation was reported by Kay et al in their paper of 2005 and it is the orientation that has been used in the simulations that are shown in other figures of the paper and the supplementary information. The wording in the figure caption of Figure 1 was changed to make this point more clear.

• The orientation selection map is only informative if the simulation of the Q band spectra is accurate, which cannot be judged here

The simulation of the Q-band EPR absorption spectra has been added to the inserts of the figures in the new version of the manuscript.

EDNMR:
• The accuracy of the frequencies given in the EDNMR figure is highly questionable given the broad features in the experimental spectra
The referee is right, the experimental features are broad and the frequency values given in the figure are subjected to some uncertainty. Therefore, we decided to show the frequency values in the figure with no decimals.
In any case, the frequencies in these spectra are used to give a first estimation of the hyperfine values which are then refined using HYSCORE results. The accuracy of the coupling parameters is determined by simulating the HYSCORE experiments.

• The orientation map is given with a + to – scale. There are no negative orientations
The + and - signs refer to more populated orientations and less populated orientations. We explain this in the current version of the manuscript.

• In panel b, the orientation map is cutting into the experimental EDNMR spectrum
The figure has been corrected so this does not happen in the new version.

HYSCORE
• Authors give no excitation bandwidth, in contrast, it is given for EDNMR after the revision
The excitation bandwith in HYSCORE experiments is 42 MHz since the excitation pulses are much shorter. This is now mentioned in the text.

Spin Hamiltonian:
• There is still an error in equation 1: I_j>1/2m nit I_i
The error has been corrected in the new version.

Further Comments:

• Figure 5 still talks about the CW spectrum.

The figure caption has been changed and now it reads “EPR absorption spectrum”

• The maximum of the EPR spectra significantly differs between the experiments and it is not clear why

The experiments were performed at slightly different frequencies.

REFEREE #2

The authors are greatly acknowledged for the effort they put into the review process to address the reviewers' comments.
We thank the reviewers for thoroughly revising our manuscript. We acknowledge the joint effort is benefitting the quality of the article.
The following further corrections are advised:

- Line 176: "Samples with a protein concentration of 400-800 mM". mM or µM? Please, check.
Thank you for realizing. There was a mistake in the "correction" in the first round. The actual concentration of the protein is 400-800 µM in a 50 mM MOPS buffer. Both mistakes have been corrected in the new version of the manuscript.

- Line 211: *db --> dB
This typo was corrected in the manuscript.

- Line 212: "The separation between the two pulses was tau = 1.5 µs". Between raising edges or between the falling edge of the HTA and the raising edge of the pi pulse? Please, clarify.
The time separation between the pulses is 1.5 µs between falling edge of the HTA and the raising edge of the pi pulse. This has been specified in the text.

- Line 232 (equation (1)): "sum over I(j)>1/2" rather "sum over I(i)>1/2".
Thank you for spotting this previously uncorrected mistake. It is now fixed.

- Line 242: "separate axis": unique axis?
We chose the wording *distinct axis* to make it more clear.

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- Line 255: "C = h/(g_e·mu_B)" rather than "C = (g_e·mu_B)/h".
The referee is right, the mistake has been corrected.

- Line 312: *isoaloxacine --> isoalloxazine.
The spelling was corrected.

- Pages 15-16: Figure 5a appears to have been introduced twice.
That is true, it has been removed now. Thanks for noticing.

- Line 469: "Spectrum taken at the *tail of the EPR absorption" --> "Spectrum taken at the high-field tail of the EPR absorption".
Thanks for the suggestion, the sentence has been changed.

- Line 498: *isoallosazine --> isoalloxazine.
The spelling was corrected.

- Lines 542-545: the new sentence starting with "The overestimation in the 13C(4a) calculations" is not entirely clear to me. I would be grateful if the authors could consider reformulating it.
The sentence was changed and now reads: "The overestimation of the isotropic hyperfine coupling of ^{13}C(4a) in the calculations is quite significant. While the

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magnitude of the calculated isotropic couplings of ^{13}C(4), ^{13}C(5a) and ^{13}C(10a) is comparable to the one of ^{13}C(4a), the experimental hyperfine values reveal that the coupling with ^{13}C(4a) is nearly half of the others."
We hope it is clearer now.

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- Line 580: *isoalloxacine --> isoalloxazine.
The spelling was corrected.

- Caption of Figure S4: the current text reads "HYSCORE simulation of [15N-FMN]-Fld variant at the high-field edge of the EPR spectrum". Please, check if the spectrum relates to the absorption maximum instead (see e.g. Figure 5b).
This was another mistake spotted by the referee. It is now corrected. Thanks!