Final response to discussion manuscript mr-2025-17

Dear Editor, dear Referees,

We are happy to enter in the discussion with the reviewers, whom we thank for their careful study of the manuscript and insightful comments. We have answered all the points mentioned by the reviewers, as shown below. I am aware that many of the points came up because I neglected the necessary diligence to consider important aspects, include all necessary information and write the manuscript clearly to avoid misunderstandings. For that I take the full responsibility and apologize to the reviewers for this neglect.

An important aspect raised by reviewer 2 is that the DEER traces after background correction, in Fig. 2 main text and in Fig. SI4, have artificial extra noise, because they were taken from the output of DEER analysis after validation, so they contain the additional noise added during the validation process. In this document, we show the proper data, alongside the figures that were contained in the original manuscript. Evidently the original data have a lower noise level than those shown in Fig. 2 main text and Fig. SI4, so the impression of the reviewers about "poor signal-to-noise ratio" is due to this mistake of ours. This is shown in the Figures appended at the end of this document, from p. 14.

We also acknowledge that the present outline of the manuscript does not do justice to the readers of Magnetic Resonance, a specialized audience that needs to be given proper background on the technical aspects. Therefore, the introduction should have summarized the background of eq. (1) and described in how far the equation applies to the problem at hand, for example. Also, a critical assessment of the factors described in the SI should have been given in the main text of the manuscript. Therefore, we will rewrite the respective sections and revise the outline of the manuscript. This will include all points discussed in the point-by-point response to the reviewers.

Further, a general point that should have been stated in the manuscript submitted was missing:

> There is no crystal structure of the cage-compounds. This should have been stated clearly in the manuscript. The structures shown as model 1 and 2 are from Molecular-mechanics (MM) geometry minimization using Spartan's built-in force field. More advanced calculations were performed on the square and triangle windows before using a combination of DFT and MM, as described in ref. 5. The entire structure was optimized with only a simple force-field model, as the computational effort required to perform an entire DFT calculation was too large. Lacking a refined geometry around the Cu-centers, it is not possible to derive even approximate g-tensor directions, and thereby determine the relative orientation of the g-tensor directions of pairs of Cu-ions in the cage to determine in how far they can be excited under the conditions of the experiment.

We describe the consequences this has for predicting the effects of orientation selection in the separate section at the end (from p.12) of this point-by-point response headed:

" I Expansion of discussion on effects of orientation selection. "

Where we also added a comment on the Cu2 reference compound, headed:

“II Analysis of the Cu2 reference compound”

In conclusion, the submitted manuscript had significant shortcomings, which we regret, and we really would appreciate to be given the opportunity to rectify this.

With kind regards,

Martina Huber

Point-by-point response to referee comments, our answers are in red.

Referee 1

 This manuscript extends on an earlier publication of the authors (Chem. Sci., 2023, 14, 11840; Ref. 5). The authors remeasured DEER data for two Cu(II) cage compounds and supplemented them by a measurement on a model compound with two copper centres. Then they analysed modal distances in distance distributions and modulation depths in DEER time-domain data and compared these parameters to expectations for different models for the cage compounds. The results are inconclusive. Given the limited quality of the experimental data (length of the traces and signal-to-noise ratio), it remains unclear whether the inconsistencies are caused by a simplistic physical model or by differences between crystal and solution structure of the compounds. As detailed below, it is likely that better quality data could have been obtained and that a better model for the spin system could have been designed. In its present form, the manuscript is not suitable for publication as it does not constitute a significant advance beyond Ref. 5.

Major criticism:

1. The measurement parameters appear to be far from optimal. Why did the authors measure at 20 K if T_1 was still that short? The asymptotic low-temperature maximum of T_m for such compounds would be expected between 6 and 15 K, probably at the lower end of this range. Because achievable maximum dipolar evolution time and signal amplitude at given evolution time depend roughly exponentially on T_m, a too high measurement temperature can have a large effect on data quality.

Response: Of course, any lengthening T_2 (T_m) is welcome, but according to Fielding et al. (Journal of Magnetic Resonance 179 (2006) 92–104) for Cu(II) there is hardly any change in T_m between 10 and 20 K. Also, so far, most Cu-Cu DEER experiments published are performed at 20 K, so it does not seem likely that there would be a large impact.

2. It is standard to perform such DEER measurements in deuterated solvents in order to prolong T_m. In this case, it is clear that the effect on achievable maximum evolution time and signal-to-noise ratio is huge, as one can compare the data for the two-spin model compound to those presented in Ref. 31. It is true that perdeuterated butyronitrile (not butylnitrile, as written in the manuscript) is a bit expensive, but compared to the total

expense of the project this is a price worth paying for improved data quality. This applies in particular when data quality without deuterated solvent is insufficient for drawing firm conclusions, as is the case here.

Response: The referee is absolutely right, the protons in the solvent could have a strong impact on the T_2. At the time, the synthesis and handling were still being optimized and we did not know if to expect DEER at all, given the many Cu(II)-ions in the systems. So the first priority was optimizing the samples, and their stability.

3. It is somewhat confusing that the authors do not use the nomenclature of Ref. 31 for the **Cu2** model compound. According to Fig. SI2, it is the [Cu-TAHA] – [Cu-TAHA] ruler **3** of that paper (see Fig. 1 of Ref. 31). Because of the distributed exchange coupling, this compound is not a good modulation-depth standard for DEER. Conformations with large coupling do not contribute to modulation due to lack in excitation bandwidth. Instead, ruler **4**, $\mathbf{2}_3$, or $\mathbf{1}_5$ should have been used.

Response: First about the nomenclature: In ref. 31 a large series of rulers was studied, so a systematic nomenclature was chosen that avoids confusion between the different molecules. As authors of the present study, we came to the conclusion that labelling the compound as ruler **3** is likely to cause confusion rather than bring clarity. However, the referee is completely right that we should have clearly stated which of the rulers from ref. 31 we use, and to do so in the main text, rather than only showing the structure in the SI. This we will do in the revision.

We agree with the reviewer that first and foremost a more detailed description of the Cu2 DEER results is needed. The choice had more to do with availability, at the time, our focus was on a compound with a suitable distance. About the exchange interaction: We had not properly realized the importance of the exchange interaction in the rulers. We discuss this point in the section (“II Analysis of the Cu2 reference compound”) on p 12 of this document, and of course in the revised version.

4. With excitation bandwidth being an issue, also for the cage compounds **Cu6** and **Cu8**, the choice of pulse lengths is not good. An optimal experiment would minimize the length of the longest pulse among both the observer sequence and the pump pulse. With the hardware available to the authors, a maximum length of 24 ns (instead of 32) should have been possible, probably even 20 ns, depending on actual power of the TWT amplifier.

Response: We thank the referee for carefully looking into our experimental parameters. Unfortunately, his/her view of our instrumentation is a bit too optimistic. The pulse parameters we use are the shortest pulses possible to obtain the required flip angles, which is our standard procedure for setting up a DEER experiment. Perhaps our TWT is the problem. Note that its nominal power is 100 W, not 130 W as in the reference mentioned under “minor points, 3. “

5. The authors state that they cannot assess orientation selection, because they do not know relative orientation of the g tensor principal axes systems (PASs). Why didn’t they try DFT computations? The software is free, a relatively small basis set allows for getting orientation right, and it would have been possible to compute only cutouts of the whole cage compounds, because spin population is localized.

Response: The referee is completely right, in principle, modern DFT calculations are certainly able to provide reasonable g-tensor directions, if the ligand environment of the Cu(II) center is known in sufficient detail. This is not the case here, and we should have made this more clear in the present manuscript (see above). As described in ref 5, it was not possible to obtain crystal structures of the complexes. The models shown derive from MD modelling (for details, see above). In these structures, the ligand-location is not sufficiently defined to derive g-tensor directions. One indication is that the Cu-ligating-atom distance varies by around 0.3 Angstrom for different centers. This is not a problem for the overall structure of the cage, but poses limits on how far the Cu(II)-ligand environment can be used for g-tensor predictions. Therefore, a full structure-refinement, preferably with a quantum-mechanical method would be needed. This was outside the scope of the present work.

We take full responsibility, however, for the fact that we should have described this clearly in the manuscript and accept that this reflects indeed a shortcoming of our description in the manuscript.

6. Even without DFT computations, it is clear that the PASs opposite Cu centres (diagonals) must coincide in ideal geometry, as they are related by an inversion centre. With the given pump and observer frequencies, contribution of the cube diagonals in **Cu8** should be at least as large as the one of the other distances. Hence, orientation selection cannot be the reason for the expected peak to be missing.

Response: We thank the referee for pointing this out and fully agree: The inversion-center related Cu-ions must have collinear g-tensors, in ideal geometry, so that is true for the Cu-centers on the cube-diagonals. While, for Cu6, this could contribute to the larger intensity of the longer distance relative to the shorter distance, it indeed does not explain the absence of the longest distance peak expected for Cu8. We will take this into account in the revision and also have expanded on the subject of orientation selection in this response to the reviewers comments, see text on p. 12, headed: “I Expansion of discussion on effects of orientation selection”.

7. Error bars should be given for modulation depth and error propagation computed for the number of spins.

Response: The referee is fully right, this should have been described in the manuscript and we will add it in the revised version. In short: The noise amplitude of compound (Cu6 or Cu8) and the reference were added. The number of contributing spins was calculated from the two extremes of the spread, resulting in the uncertainty of the number of spins: N_{spins}=2.9±0.3 (Cu6) and $N_{spins} = 2.9 \pm 0.4$ (Cu8).

8. Only the cage models corresponding to the crystal structures are clear. The authors do not exactly specify the alternative models that they consider.

Response: Sorry again, our mistake: Model 1 and model 2 for Cu6 and Cu8 stem from the same modelling software, they represent different structures with similar total energies (see above).

9. In the current manuscript, for **Cu6** the two expected distances have the same peak amplitude in the distance distribution (Fig. 2C), which is unexpected. In Ref. 5, where data

on the same compounds were measured with the same settings, the shorter distance gave rise to higher amplitude, matching expectation at least semi-quantitatively (Fig. 8B of Ref. 5). Why the different results? Given the aim of the current manuscript, this ought to be discussed.

Response: The referee is fully right, it is not explained in the manuscript, but the previous result, is fully explained by the worse signal to noise of the ref. 5 measurement. We checked this by adding noise to the data presented in the manuscript submitted here. This will be discussed in the revision, of course.

Minor criticism:

1. Not all measurement parameters are specified. For instance, what was the shot repetition time? What phase cycle was used? What exactly were t_1 and t_2?

Response: The referee is completely right, and we apologize for this omission. The text we will add is:

"The delay between the first two pulses (t_1) is 140 ns, which is incremented in eight steps of 8 ns to suppress the effect of proton modulation; t_2 was 2248 ns. A phase cycle of +x followed by -x was applied on the first pulse. The shot repetition time was 96 µs."

2. The relaxation data reported in Table SI1 are identical to the ones in Ref. 5. This can hardly be coincidence, the data is very likely from this earlier paper. This needs to be mentioned. It is also somewhat problematic, because other measurements were repeated and the samples are not long-time stable (and were probably reprepared).

Response: The referee is completely right, the data were taken from ref. 5 and that should have been stated, we apologize for this. During setting up for the DEER experiments we did not observe significant differences between the present and the previous measurements, so there were no indications for drastic changes in relaxation properties. The samples were from the same preparation batch, kept in liquid nitrogen between measurements.

3. It is not true that the setup used by the authors would not have allowed for testing orientation selection (claimed in the SI on p. 3). Essentially the same setup was used in https://doi.org/10.1002/1521-3757(20021018)114:20<4063::AID-ANGE4063>3.0.CO;2-V, where orientation selection was observed.

Response: We are confused about this remark. The molecule investigated in the reference given (Narr et a. Angew. Chemie) is a nitroxide-Cu(II) system, and indeed there orientation selection was tested, by setting the pump pulse on the nitroxide spectrum and the observer pulses at two different positions in the Cu-spectrum. In that system, the narrow nitroxide spectrum confers a high efficiency for the inversion of the pumped spins, and therefore a higher modulation depth in the DEER traces. Thereby, sufficient signal-to-noise in the DEER traces in the parallel region

of the Cu-center is obtained. For Cu-Cu systems, the inversion efficiency is significantly lower, explaining why this is not possible for the Cu-cages.

4. l. 93-95: “Contribution of a known instrumentation artifact at 1940 ns”. This reviewer does not know such an instrumentation artefact. It rather looks like the pump pulse running into (and through) the last observer p pulse. Not knowing t_1 and t_2, I cannot be sure, though.

Response: Indeed, although, nominally, the timing is such that there is no time overlap of the pump pulse with the last observer pulse in the sequence, (see response to minor point 1 of this reviewer) it is most likely a cross talk between the pump pulse as it approaches the last observer pulse that causes the artefact. See also response to referee 2, minor point, line 93 .

5. l. 173/174 “For model 2, the same intensity ratios are expected as for model 2, as long as it can be considered an almost ideal geometrical body”. Something is wrong here.

Response: Sorry for this, of course it should have been “For model 2, the same intensity ratios are expected as for model 1, as long as it can be considered an almost ideal geometrical body”. I apologize for dismal proof reading.

6. SI p. 3: “The DEERNet option to do Tikhonov analysis” does not make sense. DEERNet does not use Tikhonov regularization.

Response: Indeed, this remark is wrong, it will be changed in the revised manuscript.

7. “that a smaller regularization parameter selected in the user-adjusted Tikhonov regularization smoothens out some of the features that we interpret as noise”. This cannot be the case. Noise artefacts in distance distributions are smoothened out by larger, not smaller regularization parameters.

Response: Indeed, what is meant is the regularization parameter in DeerAnalysis not alpha. Since we refer to ‘regularization parameter’ this is ambiguous. This will be clarified in the revised manuscript.

8. SI p. 4: In the Section “Modulation depth and the relative intensities of the distance peaks” insufficient excitation bandwidth is dismissed as a reason for too low intensity of the peak at the shortest distance, in Section “Why not all spins in the cages are observed” it is not dismissed but invoked to explain the data. With a 32 ns observer p pulse, I would indeed expect reduced modulation at these short distances.

Response: Again, we thank the reviewer for pointing out unclear descriptions in the manuscript. On p. 4 SI “Modulation depth and the relative intensities of the distance peaks” it is meant that the relative intensities of the distance peaks do not change – yet taking into account the “short distance “ correction does impact the modulation amplitude. This will be clarified in the revised manuscript.

Referee 2

RC2: 'Comment on mr-2025-17', Anonymous Referee #2, 20 Jan 2026 reply

This reviewer cannot support publication of this manuscript.

The quality of the manuscript is quite disappointing. I will point out some of the issues but if authors cannot be bothered to carefully proofread their manuscript, I will not compensate this.

Response: Fully agreed, and, again, as mentioned in the introduction to this response to the reviewers comments, the manuscript has a lot of shortcomings, which we regret.

The progress this manuscript makes with respect to reference 5 is minimal and does not justify separate publication. There are no new insights in the present manuscript that go beyond the state-of-the-art.

The authors should clearly define their terms. Determining of spin numbers per cluster through modulation depth analysis and effects of sum and difference frequencies on the experimental distance distribution are two very different phenomena arising from the presence of multiple spins in the nanoobject. This manuscript uses “multi-spin effects” indiscriminately.

Response: Indeed, and also in response to the next point, this is part of what is rightly criticized and what we are changing in the revision (see also our general remark above).

Equation 1 is introduced without discussion of the underlying assumptions and approximations made. Especially for a submission to a specialised magnetic resonance journal this manuscript is very thin on background and theory. The main approximation of negligible orientation correlation of spin centres is not well met here and I would not expect eq 1 to be valid in this application. In fact, I would expect very strong effects from orientation selection and very weak effects from multiple spins being excited by the pump pulse. However, the manuscript hinges on the latter and makes no plausible attempt in rationalising or quantifying the former.

Response: See also response to the former point. In the revised manuscript we describe in how far our data may violate these assumptions and describe possible consequences. Also in relation to several other comments of this and other reviewers, this point is not at all sufficiently taken into account in the discussion. As we point out below, however, there are limitations on estimating the impact of orientation selection, yet of course it was an omission to leave this factor out.

As the experiments are set-up the perpendicular component of the Cu g-tensor is selected. This means that Cu ions with the parallel component oriented along the field will not contribute to the echo nor be pumped. In an octahedron (Cu6) the opposite vertices will have inverted orientations and thus identical g-tensor orientations. This might indeed increase the modulation depth. If one Cu is in g_perp the opposite one is as well. The 4 nearest neighbours will be in an orientation 90 degrees rotated and this might match g_perp with g_par (no modulation depth) or g_perp with g_perp (high modulation depth). This might rationalise the observed 1:1 distance ration with 4:1 expected. This effect of increased modulation depth is missing from the discussion of orientation selection.

Response: We fully agree with the referee that the effect of orientation selection on the modulation depth needs to be taken into account. While it remains true that in the absence of information on the orientation of the g-tensors with respect to the symmetry axes of the cage, orientation effects can not be determined quantitatively, the inversion symmetry of the diagonals can be taken into account qualitatively, as described by the referee here. Instead of breaking up these points amongst the responses to reviewers, we have added a detailed description to this point-by-point

answer to the referee comments see “I Expansion of discussion on effects of orientation selection”, on p.12.

The SNR is not given but likely too low <10:1 to quantify complex distributions with multiple distance populations and different peak widths (how were peak ratios determined?). This any detailed analysis is likely leading to data overinterpretation.

Response: About the SNR: See comment above and additional figures on p. 14 and 15. The peak ratio was determined by integration, so the peaks were integrated and the integral value used to determine the ratios, in order to take different peak widths into account. We are adding this information in the revision. And, of course we will add the confidence margins resulting from this in the revised version.

The modulation depth analysis pivots on the calibration with model system **Cu2**. This is actually not a good model system. If the excitation of a 16 ns pump pulse in the Cu spectrum is estimated, you would expect anywhere around 3-7%. That this is much higher here hints at orientation selection and the molecular structure (likely again inversion symmetry between the centres) matching g_perp with g_perp. If this is true, the present calibration is not robust. This is further evidenced by the distance distribution not reflecting what is expected. This will be an effect of exchange interaction and strong orientation correlation effects. Either of which would make this a poor model system the combination only makes it worse. I would recommend a calibration sample that shows the expected distance distribution to start with.

Response: Indeed, the choice of reference compound was not optimal, see also response to reviewer 1’s major point 3. To not discuss the properties of the Cu2 system was a serious omission of the manuscript we submitted. There are several factors that come into play. If, indeed, the tensor directions are collinear, orientation selection would **increase** the modulation depth of the reference sample. Also, we did not discuss the distance distribution and did not take the exchange interaction into account, although we should have done. A major factor in the difference between the distance distribution shown by Keller et al., ref. 31 and the data shown in our manuscript is that our data are from X-band, not Q-band DEER. This results in a higher modulation depth on account of the smaller spectral width of Cu(II) at X-band vs. Q-band, but also in artefacts because of residual nuclear modulation, that affects the X-band data.

That means the main conclusion of low spin numbers found in the clusters is based on a flawed calibration.

Response: Taking into account this factor would mean that the number of interacting spins in the cages is larger, confirming that there are more than two spins in the nano-object. It does make quantification of the number of spins less certain, however, and therefore it certainly needs to be discussed in the manuscript, see also “II Analysis of the Cu2 reference compound” on p. 13.

In these clusters, I would expect negligible effects of multiple simultaneous excitations of B-spins in the same cluster, and this is what you find. I would further expect strong orientation selection effects, and you seem to observe them but neither test to confirm them or rule them out and do not analyse them. These are the core findings of this manuscript. Conclusions about reliable distances and tackling of multi-spin effects are overblown.

Response: These aspects are described in “I Expansion of discussion on effects of orientation selection” on p. 12, below. In the SI of the submitted manuscript, we also clearly describe that and why we were not able to perform experiments that would abolish orientation selection. Also, please see our response to the points of reviewer 1 and 2.

Minor

The references are not numbered in order of appearance

The reference section needs careful formatting – the care taken here seems to reflect on the general care taken when preparing the manuscript.

Response: Sorry for this, the writing of this manuscript is not what it should be, I apologize and am really sorry to submit the reviewers to this.

Line 31 ref 27 is given, but it should be 29

Line 82 refs 4,5 are not the most appropriate for 4-pulse DEER

Response: Indeed, the references will be completely revised in the revised version.

Line 93 The “known artefact” should be explained. It could be crossing the refocusing pulse or a crossing echo by detection and pump pulses.

Response: We should have phrased this differently: It is an artefact of our instrument that we know about. We agree with the reviewers suggestion that it is due to the refocusing pulse, and most likely a cross talk of the refocusing pulse with the pump pulse, as it does not depend on the echo and occurs at a fixed time before the refocusing pulse. Since nominally there is no overlap between pump and refocusing pulse, it must be a reflection in the system, see also response to reviewer 1 minor point 4.

Line 107 is the concentration of **Cu2** 0.6 mM or the Cu concentration?

Response: The concentration of the Cu2 sample given refers to the ruler concentration, we are specifying this in the revision.

There is main text between Table 1 and its header.

Response: Thank you for pointing this out, this is changed in the revised version.

SI: “So in this case, the advantage of the DeerNet program, namely that processing of baseline and form factor is user-invention free [7, 8], cannot be fully harnessed” Do you mean intervention?

Response: Yes, indeed.

SI page 3 states a DEERNet option of Tikhonov analysis. As this is not an option in the software this needs clarification.

Response: True, and we changed this in the revised manuscript.

SI page 4: Due to the overall low signal-to-noise ratio of the data we did not expect unambiguous solutions.” It would be helpful if this assessment of the reliability had made it into the main text.

Response: A very good point and it was not meant to “hide” this point, in the revised version, this will be discussed in the main text, as part of the new outline.

Figure SI.4 The raw data (A) shows no noise but very strong ESEEM-like modulation on top of some typical DEER form-factor. The background corrected data (B) has much stronger noise that masks the high frequency modulation (if it is still there). This data is clearly not just background subtracted (and cut). The noise seems also to increase upon background correction in Figure 2 (main text). Are these the forma factors after validation with added noise?

Response: We are grateful to the reviewer for alerting us to this. Indeed, the traces shown in the figures (Fig. 2 of the main text and Fig. SI 4) show the traces with added noise, because they were taken from the analysis after validation. In the attached Figures, we show the proper data that should have been in the figures.

Referee 3

In this manuscript, the authors apply DEER spectroscopy to two relatively complex copper clusters, referred to as Cu6 and Cu8, both of which are of interest as catalytic systems. The stated aim is to extract inter-copper distances using DEER and to build upon multispin effects. The problem is introduced reasonably well; however, the experimental results are not sufficiently convincing to support the conclusions drawn.

A central part of the discussion focuses on the modulation depth. Unfortunately, the signal-to-noise ratio (SNR) of the DEER data is low. For example, in Figure 2B, the normalization appears unreliable given the noise level. While these experiments are undoubtedly challenging—particularly in light of the short phase memory times (T_m) reported in the Supporting Information—the current data quality does not allow robust conclusions to be drawn.

Response: See comments at the beginning of this document, the Figures shown below and our response to the last minor point of referee 2. In short: The data after background subtraction are fraught by the extra noise added in the validation process, and therefore carry extra noise (Fig. 2 of the main text and Fig. SI 4). In the attached Figures, p.14 and 15, we show the proper data that should have been in the figures.

Significant effort is devoted in the Supporting Information to addressing some of these issues. However, the Supporting Information contains details, particularly regarding orientation selection and its potential impact on the relative intensities of the detected spin pairs, that should be discussed more explicitly in the main text.

Response: This is a very valid point. See our remarks above, before the start of the point-by-point response.

For the Cu8 system, the SNR is particularly low, and it is therefore not possible to draw reliable conclusions from the fits. As a result, the proposed discussion of structural flexibility in Cu8 is not convincingly supported by the experimental evidence presented.

Response: About the SNR, please see our response to the first point you mention. Further, we agree that this point is not well covered in the submitted manuscript. There are several reasons why we come to this conclusion, and we will make this clear in the revised version.

In view of these points, and considering previous related work (https://pubs.rsc.org/en/content/articlelanding/2023/sc/d3sc03745b), it is unclear what new insight the present study provides. I therefore cannot recommend the manuscript for publication in its current form.

Response: We fully agree that the current form of the manuscript has significant shortcomings. We are confident that the revision we outline here solves this.

Minor comments

Equation (1) would benefit from a clearer introduction, including an explanation of its domain of validity. In addition, it does not appear to be used elsewhere in the manuscript.

Response: Yes, indeed, as pointed out in our response to reviewer 2, a more in-depth description of this equation is needed. This will also cover the point made by the present reviewer, namely that the equation is not properly explained, and it will be discussed in the revision.

The origin or the artifact at 1940 ns could be explained.

Response: see response to reviewer 2, line 93, and referee 1 minor point 4.

Given the large g-anisotropy of Cu(II), and based on the models employed (which could be reported in the main text), the authors should discuss how orientation selection is expected to influence the observed modulation depth.

Response: We fully agree with this point of the reviewer and will expand the discussion of orientation selection in the SI with a detailed discussion of how the g-anisotropy affects the modulation depth.

Additional material for point-by-point response

Part 1: Additional discussion points

I Expansion of discussion on effects of orientation selection

In addition to the discussion in the SI of the submitted manuscript, here we discuss the aspects related to orientation selection that were brought up by the referee comments. In the revised manuscript, these aspects will be combined in a new version of the section on orientation selection in the SI, and the main conclusions will be evaluated in the main text.

The relative orientation of the g-tensors of the Cu(II)-ions in the Cu-cages are not known, as there is no X-ray structure, and the local structure around the Cu-ions obtained by the modelling approach is not sufficiently defined. Therefore, the amount of orientation selection under the experimental conditions of the DEER experiments cannot be determined, except for one symmetry element of the cages, the symmetry that relates the two diagonally opposed corners of octahedra and cubes. We will add a figure to illustrate this, for now we just state that the pairs concerned are those with the longest expected distance in Cu6 and Cu8. Irrespective of the orientation of the g-tensors relative to this diagonal, the tensors should be co-linear, leading to simultaneous excitation of the Cu-pairs lying on that diagonal. For the other Cu-pairs in the cages, orientation selection is likely and would reduce their contribution to the DEER curves. As a result, these Cu-pairs would be underrepresented in the DEER response.

This has two consequences:

(i) The relative intensities of distances would differ from the theoretically expected (see Table 1 main text) favouring the pairs located on the body diagonals. This agrees with the trend in the distance-distribution observed for Cu6, Fig. 2 main text, but disagrees with the one found for Cu8, where the longest distance is not observed.

(ii) The modulation depth would be smaller, insofar as the pairs that are not on the diagonal are not fully excited. This was not discussed in the manuscript submitted, however, it is clearly relevant.

II Analysis of the Cu2 reference compound

The submitted manuscript neglected to discuss how the properties of the Cu reference compound (Cu2) affect the conclusions drawn. The two main aspects are:

(i) The possibility of orientation selection. The rigid structure of the linker could impose co-linearity of the g-tensors of the Cu-pairs, which would lead to increased modulation depth.

(ii) The presence of the J-coupling between the Cu-centers was not taken into account in the analysis of the data.

As both factors can change the modulation depth, they have to be included in the discussion of the number of spins observed for the Cu-cages. For point (i), in principle, a larger modulation depth would result than for random orientations of the g-tensors, so it would not contradict the main

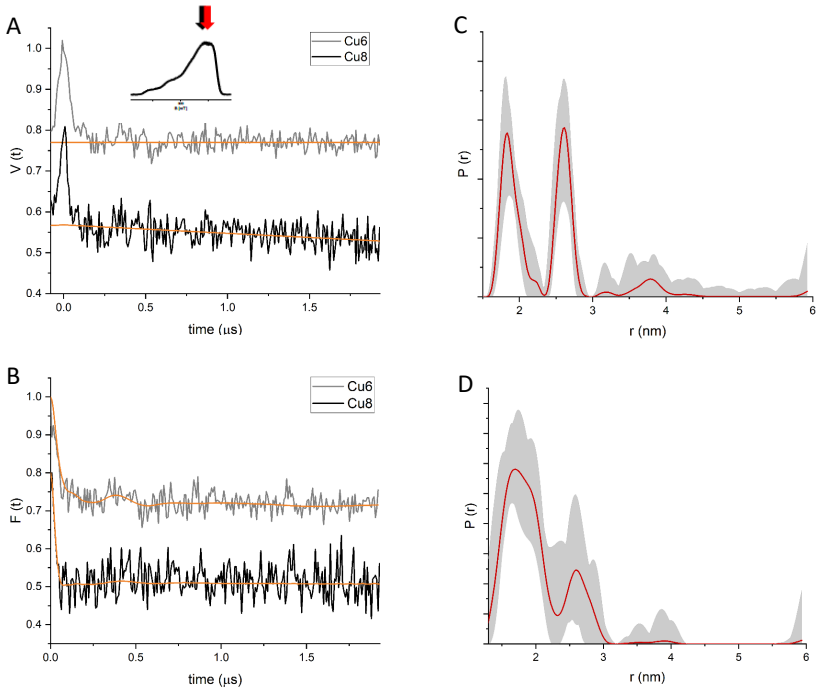
conclusion of multi-spin interaction in the Cu-cages. To take (ii) into account, we will properly analyse the distance distribution of the Cu2 compound, which we should have done in the first place.

Of course, we will describe the consequences that all the above points have for the conclusions of the manuscript in the revision.

Part 2: Additional figures

Here we show the comparson between the data shown in the submitted manuscript and the data that have the original noise level (explanation, see beginning of this document).

Fig. 2 in submitted manuscript, main text:



Proper panel for Fig. 2B:

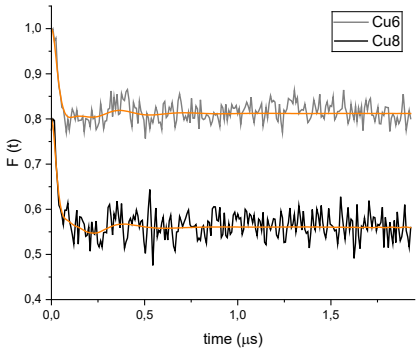
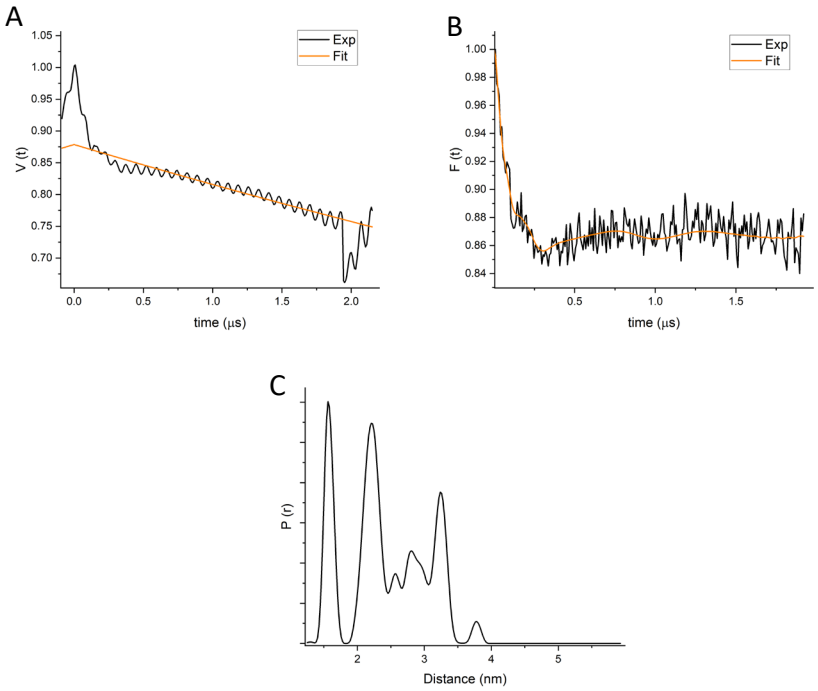


Fig. SI 4 in submitted manuscript SI



Proper panel B for Fig. SI 4 B