POINT-BY-POINT REPLY TO REVIEWERS´ COMMENTS

Analysis of the electronic asymmetry of the primary electron donor of photosystem I of *Spirodela oligorrhiza* by photo-CIDNP solid-state NMR
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REVIEWER GUNNAR JESCHKE

We thank the reviewer for helpful comments.

1. You may want to state that the 2020 Artiukhin *et al.* work uses a frozen density embedding approach for improved treatment of the PS-matrix interaction.
We added the statement to the reference (page 4, bottom).

2. I understand that 'bidirectional' was introduced before as a term for what actually is 'two-sided' electron transfer. However, I find this extremely confusing. In all other science and engineering, 'bidirectional' means 'forth and back', which is not what a PS should do under normal condition. It adds to the confusion that in your experiments, with reduced F_x, ET becomes bidirectional in the usual sense of the term. There is precedent on using the proper term 'two-sided' (https://doi.org/10.1529/biophysj.105.059824). Please consider using it, too.
We thank the reviewer for this helpful advice and changed the wording accordingly (Page 5, lines 87-95 and page 7, line 154).

3. It is not clear to me how exactly you referenced ^{13}C shifts (p. 9, l. 7). Do you quote values with the chemical shift of chlorotrimethylsilane set to 0 ppm or do you set TMS to 0 ppm and assume a known shift for chlorotrimethylsilane? If it is the latter, which shift do you assume? It is about 0.4 ppm difference to normal convention.
In fact, we took chlorotrimethylsilane as a reference by mistake, and would like to thank the reviewer for pointing our attention to this issue. We now calculated the ^{13}C shielding for TMS with the same methodology, which results in an offset of 9.37 ppm with respect to the value calculated for chlorotrimethylsilane. This is considerably larger than the value of 0.4 ppm mentioned by the reviewer.
But (i) since we speculate that this is more probably due to difficulties in our (non-relativistic) calculation on chlorotrimethylsilane, (ii) it only adds a constant shift to all calculated values presented here, which in no way changes any of the conclusions, and (ii) it actually brings the calculated values overall in better agreement with experiment, we decided to switch to the TMS shielding as a reference. This is now also consistent with experiment.
Therefore, we changed: the Materials & Methods section (page 9, line 207) and in the SI section 2.3 (page 6, first para).

4. It is imprecise to state that labeling has an influence on the photo-CIDNP mechanisms. It does have an influence on the outcome, i.e., on the observed nuclear spin polarization.
That is true. Page 12, line 263: We changed "influence on mechanisms" to "influence on spin-dynamics".

5. I am not sure about the interpretation in terms of relative contribution of the TSM and DD mechanisms. If isotope labeling changes lifetime(s) of the radical pair, the TSM polarization will also be affected. If it does not, DD should not be suppressed by such labeling. You might want to state that your explanation is tentative.
We added a statement limiting the interpretation to samples at natural abundance (p. 12, line 268).

6. That the polarization of the aliphatic carbon at 52 ppm vanishes at low field implies that polarization transfer by spin diffusion to this carbon is negligible at low field, but not high field.

It does not strictly imply that the neighboring aromatic carbons do not obtain enhancement. This should also be formulated with more caution.

Page 12, line 275: We changed "implies" to "might imply" and agree that it is smart to do not rule out other effects.

7. Conclusion: "Our study contributes to converging and convincing evidence" Please leave it for the readers to decide whether the evidence is convincing. It may be also useful to discuss current limitations. The "which is thought to originate" on page 16 reveals that there is no quantitative understanding (yet) of the supposedly dynamic origin of the asymmetry in bacterial reaction centers. It is also somewhat dangerous to draw conclusions on effects of static electronic structure from only ground-state properties. That chemical shifts are similar between the two types of PS in the diamagnetic "resting state" does not necessarily imply that the electronic structure of the donor and acceptor radical states is also similar.

We changed the wording: "Our study contributes to converging and convincing evidence" is now: "Our study suggests". We agree that chemical shift information does not allow to conclude on excited-state properties. DR intensities obtained at the right field might do but we do not want to overstretch the present discussion.

8. Please number pages in the Supplementary Material.

Done.

9. Are you sure that a *short* MD calculation would be sufficient to improve chemical shift computations? In other words, can you exclude that chemical shift changes on longer timescales? Rather long MD trajectories would still correspond to the fast chemical exchange limit in NMR.

The short MD helps with the assessment of the QM optimized structure, because large structural deviations between the MD and QM structures indicate that the structural ensemble is significantly different from the QM single point geometry. We agree that effects happening on longer time scales are not covered by a short MD. A comment on that was added in the manuscript. (page 11 bottom)

10. The title does not appear to reflect your main conclusion

Thank you very much for point this out. We changed the words "electronic asymmetry" to "electronic structure".

Typos/grammar:
p. 2, Line 32, comma after 'properties' is superfluous
p. 3, Line 47: 'Synechococcus elongates' should be typeset italic
p. 4, Lines 67-69: Please be consistent with notation of PA and PB (either always or never subscript)
p. 7, Line 47: superscript missing in '13C'
p. 12, line 60: Please do not jump forth and back between fields and frequencies.
p. 18, line 37: "similar extend" should read "similar extent"
SI, Section 2.5: "calculalted" should read "calculated"

All corrected. Thanks.