

## 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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This manuscript uses the technique of photo-CIDNP MAS NMR to show that  $^{13}\text{C}$  chemical shift differences between cofactors do not correlate well with the symmetry/asymmetry of electron transport in photosystem I from duckweed. This result provides indirect support for the hypothesis that differences in molecular dynamics and electronic excited state properties are responsible for breaking the functional symmetry of the reaction centre.

### Specific comments

1. The Materials & Methods section gives rather little information on the procedures the authors have developed to incorporate  $^{13}\text{C}$ -labels into PSI particles. For example what does “exposed” in “plants were exposed to  $\delta$ -aminolevulinic acid” (line 160, page 6) actually mean? Given that this is the *first* time that anyone has managed selective incorporation of  $^{13}\text{C}$  isotope labels into PSI from duckweed, I think there should be a little more detail on how this was achieved so that others will be able to do similar experiments in future.
2. We are told (page 11) that it was not possible to assign the  $^{15}\text{N}$  resonances to specific cofactors on the basis of the chemical shifts. Is there any information in the relative CIDNP enhancements (Fig. 2) that could help in this regard?
3. On page 12, it is not clear why the CIDNP enhancement of C17 must be the result of spin-diffusion. Does it have a negligible hyperfine interaction? Are there no other labelled aliphatic carbons that might receive polarization by spin-diffusion?
4. The heading of columns 3-6 of Table 1 is “Tentative assignments”. Are these the same tentative assignments that “strongly suggest” (page 13) that all four cofactors are involved in the spin-correlated radical pair and therefore that both electron transfer pathways are active? Since this is one of the main conclusions of the paper, I think there should be a bit more discussion of how it was reached.
5. I am afraid that Table 1 is a mess. Some horizontal lines to separate the entries for the different carbons would make it much more readable. For example, at the bottom of page 14 in the right-most column, there appears to be 1 assignment (coloured red) for C11 (coloured red in the left-most column) and three red assignments for C9 (coloured black in the left-most column). Given that red means 4-ALA labelled and black means literature values, either some of the colours are wrong or all four of the 9.4 T assignments actually belong to C11 and none of them to C9.
6. If all four entries at the bottom right of Table 1 on page 14 are in fact for C11, can anything be learnt from the fact that one is absorptive and the other three emissive?
7. Page 18: “This confirms that both electron pathways in PSI of duckweed are active and that the electron transfer occurs symmetrically”. Symmetrically suggests 50:50 along the two branches. If this is the intended meaning, then I’m not sure where this ratio comes from. Or is “symmetrically” being used rather loosely to mean something like “not exclusively by one branch”.

### Minor comments

Lines 47, 73, 75 : elongates → elongatus?

Line 69: PB → P<sub>B</sub>

Page 18, line 337: extend → extent