

Response to Reviewer 1 Comments

The authors have essentially improved the manuscript and have addressed most of the comments.

Point 1: Major comment to Section 3.4. Effect of TSP9 mutation on ET (electron transport).

- 1.1. Concerning comment 4: At this point some further work is needed. Fig.5 should be replaced by original data (dots + lines), which were provided by the authors in the response. These data contain measurements under **five different conditions** (and not under four, as in the original manuscript); they, therefore, contain more information.
- 1.2. The Y axis should be labeled in true units of optical density, currently there are some arbitrary units.
- 1.3. The text of this section should still be reviewed by an expert in electron transfer. The text is unclear and wrong in many places. Specifically, FNR is not located downstream of P700 in the ET chain because an electron cannot reach FNR from P700 without being photoexcited. Generally, the terms “upstream” and “downstream” can be misleading when applied to photosynthetic electron transfer chains, better to avoid them.
- 1.4. Also, it is unlikely that an electron can return from P700 to PC, as authors suggest in the revised manuscript, because the midpoint redox potential of P700 is much higher than that of PC. Furthermore, the amplitude of fast absorbance changes in response to switching the light off do not depend on whether P700 was oxidized by light (DBMIB, TSP9 at 37 C) or not. Most likely these fast absorbance changes are unrelated to the electron transfer around P700 (perhaps, electrochromism of chlorophyll of some kind or a bug in the experimental setup?).

Response 1: section 3.4 was revised.

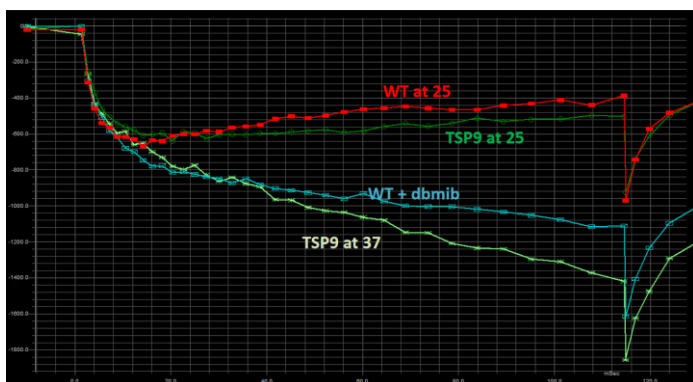
- 1.1. Fig 5 was edited in order to present the full reduction detected during the measurements. This was done by dividing the graph into two main phases: light oxidation and dark reduction. The second, which is much slower was given using a logarithmic scale to describe the time course. We hope the figure will now give more information than it gave before. However, it seems that there was a mistake in labeling the JTS curves in the original data provided in the previous revision letter. We provided two figures from the JTS output from two different experiments, but the repetitions were performed for the same conditions. The conditions we sent were the same as seen in the original manuscript (that is to say: WT at 25, WT + DBMIB, TSP9 at 25, TSP9 at 37). The "5th" condition the reviewer saw "TSP9+DBMIB" was mislabeled and was supposed to present TSP9 at 37.

Previous response:

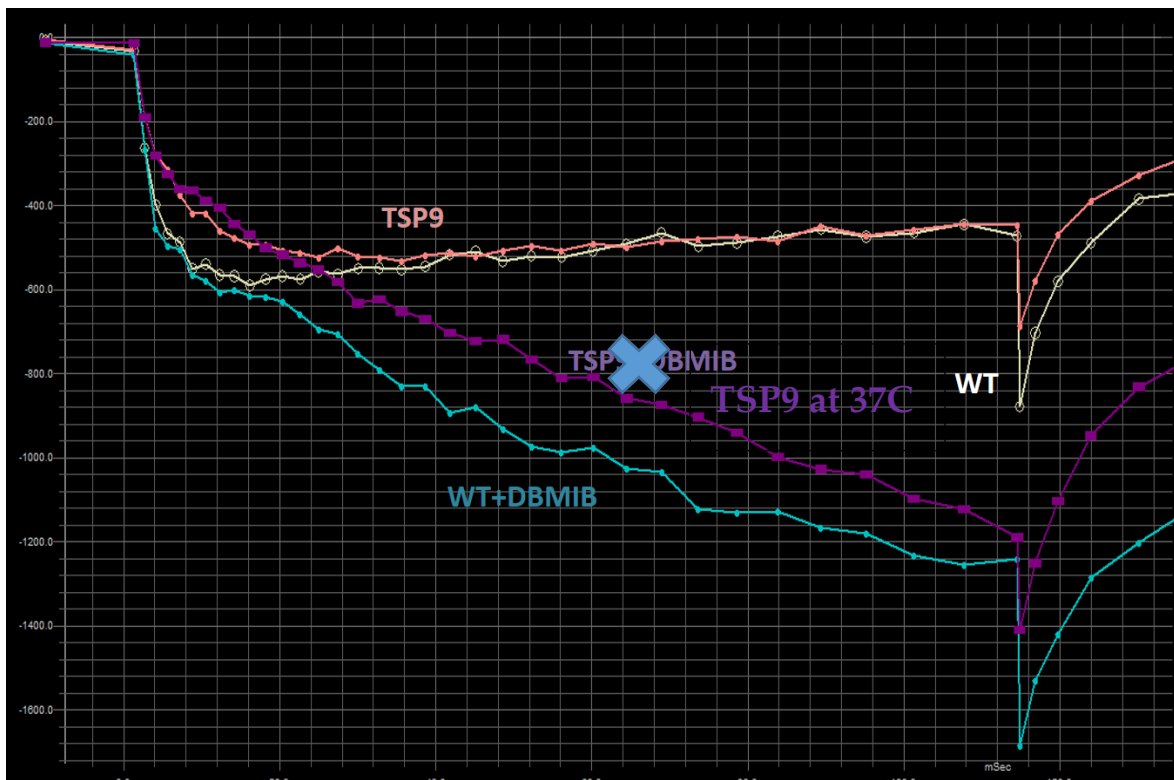
Point 4: Figure 5 and related text: someone who understands photosynthetic electron transport and its measurement should rewrite this section. As it stands, this part of the manuscript is unacceptable. The kinetic curves are hand-drawn with a marker pen; the output of the JTS-10 (Joliot-type spectrophotometer) is actually a line of dots. Even with these hand-drawn curves, it is unclear why turning off the light causes additional oxidation of P700; something seems to be wrong with the experimental setup.

Response 4: while the JTS-10 visual output is indeed a line of dots, these dots are also given in quantitative levels as a function of time. The curves shown in figure 5 were drawn by excel using the quantitative output of the JTS-10. The original curves visualized by the JTS are attached to confirm the data presented. Moreover, another repetition of this experiment is also attached in order to show these curves are not a 1-time occurrence. Since the JTS measurements were performed in vivo on full cells containing all involved proteins, the additional oxidation of p700 seen when the light was turned off is assumed to represent the electron returning from p700 to oxidized PC. When the measurements are performed in vitro on isolated proteins with the specific electron carriers PMS and ascorbic acid, these jumps in the curves disappear. the text has been revised according to the notes given here in order to clarify the curves seen.

Properly labeled:



Relabeled:



1.2. The Y axis units were changed to relative units (r.u) of the delta absorbance. The optical data of the measurement is actually the change in the absorbance detected by the JTS.

- 1.3. The text was read by a colleague in the field of electron transport and debated with. A revised text was written to accurately describe the data and previous knowledge.
- 1.4. The reaction of oxidation of P700 by PC is in equilibrium with a rate constant >0 , these means that under certain circumstances a backward reaction may occur. This is mainly derived from the fact that the potential difference between PC and P700 stands at about 100mV and the affinity of PC to P700 can change depending on their association. When intercrossed- PC-PPSI Em change in about 60mV. Although this is not common, excited electrons can also return from P700 to PC. In order to dispute the idea of electrochromism of chlorophyll, a ESC test can be performed using the JTS.

Point 2: The English language should be checked, particularly in relation to description of the subunits of PSI that lack in the structure. It seems not quite appropriate to write about them as “missing” (who is missing them?) or “omitted” (by whom?).

Response 2: note taken, text was re- written.