Peer Review File

Manuscript Title: Photosynthesis re-wired on the pico-second timescale.

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referee #1 (Remarks to the Author):

The authors use ultrafast transient absorption spectroscopy to study cyanobacteria and photosystem electron transfer. The experiments were carried out well and with appropriate statistical analysis. The methodology is solid, but it is unclear how these lessons learned will teach the practitioner in the field new information that they can use for bioenergy or artificial photosynthesis. The references to previous work are appropriate and the paper is well written although very short, so more detail could be provided to make it easier for the non-specialist to read the article, but it seems to be focused more for the specialist than the broad audience of Nature from both the length and the approach.

Referee #2 (Remarks to the Author):

Review Baikie et al, Nature April 2022

The manuscript presents a study of the ultrafast photoinduced dynamics of PSI and PSII in whole cells, and in reaction centres. The main point is that the authors propose that a quinone (DCBQ), which is used since long as an alternative electron acceptor in PSII, is able to accept an electron already from the initial excited/CT state(s) of the P680/P700 pigments, on a short picosecond time scale. This would suggest that hybrid systems could be made that extract electrons from PSII or PSI at a more reducing potential, thus allowing for more energy-rich products. I believe the result, if confirmed, would be of great general interest both for the understanding of natural photosynthesis, and for biohybrid devices for solar energy conversion. The ability to do ultrafast optical spectroscopy on whole cells is an additional interesting aspect. I believe the manuscript may become suitable for publication in Nature.

My main question is whether the manuscript really shows that electrons are extracted by DCBQ, on the time scale investigated. The authors present femtosecond transient absorption (fsTA) data after excitation of whole cells at 450 nm. They see an immediate bleach at 685 nm, and another bleach band at 715 nm growing in on a 2 ps time scale. Both features decay on the tens of ps time scale. The decay of these features is accelerated upon addition of DCBQ, which the authors take as evidence for electron transfer to DCBQ. The authors also point out that there is no spectral overlap between the absorption spectra of reduced DCBQ and the intact cells, which make them exclude Förster and Dexter energy transfer, and conclude that electron transfer must be responsible. Finally, by comparison with mutants where either PSI or PSII is inactive, the authors conclude that the features observed are mainly due to reactions in PSI.

The authors do not give references to previous fsTA spectra on PSII and PSI, to support their assignment of the bleach features to the reaction centre ground states (P680 and P700). In the SI, ref 14, such spectra for PSI are given after 670 and 700 nm excitation, and they do not show a distinct bleach at 715 nm; it is rather an isosbestic point. Similar for Blankenship and co-workers Biochemistry 1997 p 2898. I suppose PSI references are most relevant, as the authors data suggest these signals dominate. The authors must discuss these deviations and their assignment and give suitable references. It would also help if the authors added fsTA spectra at selected times (to the SI) to make it easier to observe the shape and evolution of the TA spectra.

The authors excite at 450 nm, which should initially populate one (or more) higher electronic state(s). Is it possible that some of the fsTA spectra and dynamics are related to higher excited states? It should definitely be possible that excitation and reaction with DCBQ occurs from other states located on other pigments than P680 and P700, which are transiently populated only because of the 450 nm excitation. The results and possible benefits for biohybrid systems would then be limited to a smaller part of the solar spectrum. This must be considered.

Finally, on the arguments against energy transfer quenching, there are other possible quenching mechanisms than energy and electron transfer. In addition, Dexter energy transfer does not require spectral overlap (although of course there must be acceptor states at suitable energy). The authors should consider these points. Then there is a typo in the main text and SI: the overlap requirement for Förster transfer is between the absorption of DCBQ before reduction (not reduced DCBQ) and the fluorescence (not absorption) spectrum of the photosystem.

===== End of comments =====

Author Rebuttals to Initial Comments:

Referee #1 (Remarks to the Author):

The authors use ultrafast transient absorption spectroscopy to study cyanobacteria and photosystem electron transfer. The experiments were carried out well and with appropriate statistical analysis. The methodology is solid, but it is unclear how these lessons learned will teach the practitioner in the field new information that they can use for bioenergy or artificial photosynthesis. The references to previous work are appropriate and the paper is well written although very short, so more detail could be provided to make it easier for the non-specialist to read the article, but it seems to be focused more for the specialist than the broad audience of Nature from both the length and the approach.

We thank the referee for their useful summary of our work and overall positive assessment.

Following the reviewer's recommendations, we have now significantly improved our manuscript in the following ways:

1) We have expanded our study and added methyl viologen (MV^{2+}) to our library of mediators examined. We selected MV^{2+} because it is a different class of mediator to DCBQ that has a sufficiently reducing midpoint potential (600 mV more negative than DCBQ) for reductive catalysis (for example H^+ and CO2 reduction) and it is the most common synthetic mediator used in the fields of artificial photosynthesis and fuel cells research. Through these new experiments, we could show that MV^{2+} interacts with PSI on the ultrafast timescale in the same manner as we observed for DCBQ (see SI Section 1.7). This is an exciting result that shows: **i)** the new pathway is not limited to one class of mediator molecules alone – this greatly opens up the range of potential mediators that can be explored in the future to enhance and expand on a this pathway (e.g. those that exhibit even higher reducing power or that could facilitate more efficient and/or selective charge extraction); and **ii)** that **biological photosynthesis can be linked to artificial photosynthesis** via this newly uncovered pathway for more efficient power and chemical generation.
2) We have broadened our introduction to the technical aspects of the paper. In particular, the general introduction to the spectroscopy techniques should now allow non-specialists to access the paper. This is highlighted in significant additions to the main text, particularly the beginning paragraphs and conclusion, as well as adaptions to Figure 1. We believe that these additions, along with the additional MV^{2+} experiments now provide a more generalised understanding suitable to the broad audience of Nature.
3) Motivated to connect with the practitioner in the fields of photosynthesis, (semi)artificial photosynthesis and biotechnology, we added explanations of how this study directly opens up new avenues of interrogation or can be used to push these fields forward. To further explain here:
    - For the photosynthesis community, our work demonstrates how ultrafast spectroscopy can be combined with microbiology to study the very first steps of photosynthesis **within living photosynthetic organisms** to fill knowledge gaps pertaining to photosynthesis bioenergetics. Previously, similar studies were only possible under very limited contexts (mostly isolated proteins at non-physiological conditions). The new ability to probe the early steps of photosynthesis (backed up by the use of appropriate mutants and isolated components as controls) under physiological conditions means we can now understand how light is connected to charge conversion steps more accurately than ever before. Consequently, we can now see the previously missed interactions between electron mediators and the photosynthetic electron transport chain at the early stages of photo-to-charge generation. Importantly, mediators have been used in photosynthesis research since the early stages for probing function and enhancing activity. Our results

fundamentally **challenge** our understanding of the photosystems and how their bioenergetics can be regulated. In the future, our methodology can be used to study a range of biological processes including photoprotection mechanisms that occur at early steps after light absorption and remain poorly understood.

- For the (semi)artificial photosynthesis community, we added the new MV^{2+} experiments, which was the missing link that demonstrates the applicability of this new pathway for generating power, fuels and chemicals using photosynthetic machineries. We also reiterate our previous conclusions, which is that this pathway is a way forward for obtaining the highest possible energy electrons directly from reaction centres, which can be a **game-changer for efficiencies achievable** via (semi)artificial photosynthesis. We have also added brief discussions of lessons gained about mediator design going forwards – for example, the importance of midpoint potentials, lipophilicity and cell membrane permeability, and cytotoxicity.
- For the biotechnology/bioenergy community, we added explanation of how this pathway can be used to study and even **alleviate yield losses caused by photoprotection** mechanisms, which is one of the biggest bottlenecks in crop yields and algal biotechnology.

We hope the reviewer agrees that these results are particularly pertinent for advancing research in biotechnology, biological and artificial photosynthesis.

Summary of author actions:

- Rewritten the main text (mostly in the first paragraph of the main),
- Added review references of the technique accessible to photosynthesis researchers.
- Developed a new experiment with the mediator methyl viologen and reported that it operates in a similar manner to DCBQ. This section directly guides the reader and the community i) in ways to study ultrafast processes in photosynthesis in vivo (using ultra-fast spectroscopy), and ii) to find other improved mediators which may circumvent the energy losses associated with photoprotection mechanisms and thermalisation further downstream in the PETC (see new sections: **SI Section 1.8** and the last paragraph in Results and Discussions in the main text).
- Expanded the conclusions in the main text and highlighted the broad application of these results to bioenergy, biological and artificial photosynthesis.

Referee #2 (Remarks to the Author):

The manuscript presents a study of the ultrafast photoinduced dynamics of PSI and PSII in whole cells, and in reaction centres. The main point is that the authors propose that a quinone (DCBQ), which is used since long as an alternative electron acceptor in PSII, is able to accept an electron already from the initial excited/CT state(s) of the P680/P700 pigments, on a short picosecond time scale. This would suggest that hybrid systems could be made that extract electrons from PSII or PSI at a more reducing potential, thus allowing for more energy-rich products. I believe the result, if confirmed, would be of great general interest both for the understanding of natural photosynthesis, and for biohybrid devices for solar energy conversion. The ability to do ultrafast optical spectroscopy on whole cells is an additional interesting aspect. I believe the manuscript may become suitable for publication in Nature.

We thank the reviewer for the detailed and constructive comments on our manuscript, as well as their appreciation of the wide impact of the study and its findings. We believe the manuscript is now significantly improved; importantly, we have further backed up our results to draw stronger conclusions and addressed each of concerns in depth.

The authors present femtosecond transient absorption (fsTA) data after excitation of whole cells at 450 nm. They see an immediate bleach at 685 nm, and another bleach band at 715 nm growing in on a 2 ps time scale. Both features decay on the tens of ps time scale. The decay of these features is accelerated upon addition of DCBQ, which the authors take as evidence for electron transfer to DCBQ. The authors also point out that there is no spectral overlap between the absorption spectra of reduced DCBQ and the intact cells, which make them exclude Förster and Dexter energy transfer, and conclude that electron transfer must be responsible. Finally, by comparison with mutants where either PSI or PSII is inactive, the authors conclude that the features observed are mainly due to reactions in PSI.

The authors do not give references to previous fsTA spectra on PSII and PSI, to support their assignment of the bleach features to the reaction centre ground states (P680 and P700). In the SI, ref 14, such spectra for PSI are given after 670 and 700 nm excitation, and they do not show a distinct bleach at 715 nm; it is rather an isosbestic point. Similar for Blankenship and co-workers Biochemistry 1997 p 2898. I suppose PSI references are most relevant, as the authors data suggest these signals dominate. The authors must discuss these deviations and their assignment and give suitable references.

The reviewer raises an important point regarding the spectral assignment of the fsTA. As the reviewer correctly summarises, we used isolated photosystems to probe the spectral assignment of the *in-vivo* measurements. We found that the isolated photosystem transient absorption spectra are consistent to the *in-vivo* measurements, which we took as sufficient evidence for our conclusions. However, we agree that it is pertinent to relate our observations more broadly to the significant body of preceding work on isolated photosystems (such as the e.g. work by Blankenship and others).

We wish to outline two extensions of the reviewer's argument which we will address in detail.

1. Relationship between in-vivo measurements and isolated reaction centres
2. Relationship between isolated reactions and other reported results.

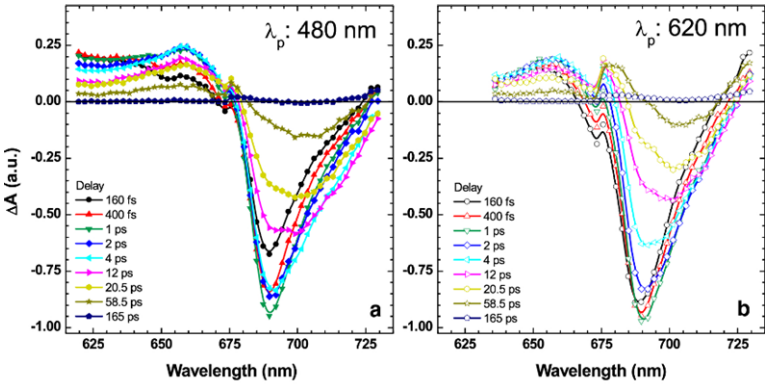
For the first point - we wish to highlight an important factor in *in-vivo* measurements. We claim that a reaction centre *in-vivo* will have meaningfully different spectral dynamics to that of an isolated reaction centre. This could arise, for example, from the local solvatochromic environment, the local protein scaffold or alternative relaxation pathways in an operational PETC. These factors among others will result in different decay pathways available to the photo-excited reaction centre, causing altered spectral dynamics. The exact biological nature underlying the change in dynamics from *in-vivo* to isolated reaction centre is difficult to pinpoint without more targeted studies and goes beyond the scope of our current work.

However, we wish to highlight that *in-vivo* measurements presented here are more relevant to physiological conditions, as well as in biotechnological and (semi)artificial photosynthetic technologies, which mainly employ whole cells as the active material. Importantly, although the dynamics may change, we expect limited changes in the bleach (initial signal) on ultrafast timescales. This is why we attempted to compare the same isolated reaction centres to the in-vivo measurements to make the spectral assignment and avoid complications arising from different preparation methodologies (see corresponding SI section). Critically, this allowed us to demonstrate that the signatures and interactions we observe are due to reaction centres, particularly as these results are supported by the cell mutants that exhibit only PSI or PSII.

For the second point, comparing different isolated reaction centres, we are using a blue pump, which is infrequently used in the literature. We chose this wavelength to avoid any interference from the pump itself, to be able to clearly resolve all ultrafast features of interest at early timescales. However, in an effort to benchmark our results to the wider literature, we conducted a more thorough literature

review on transient dynamics of PSI, which is the main signal source in our study as verified through internally consistent controls.

We draw the reviewers attention to a recent study by Maiuri and Santabarbara (DOI: 10.1007/s11120-020-00717-y)[1], which investigates the ultrafast excited-state dynamics in land plants Photosystem I LHC complexes as a function of wavelength. The ultrafast transient spectra after excitation at 480 nm (close to 450 nm excitation used in our study) are strikingly similar to our spectra and dynamics, characterised by two negative peaks at 680 and 715 nm. Furthermore, these spectra are consistent with many other literature reports, which we have now included in the main text for traceability with the inclusion of a new paragraph.



Review Response Figure 2 - Transient absorption (TA) spectra of the PSI-LHCI supercomplex recorded at selected pump-probe delays, as indicated in the legend, upon excitation at 480 nm (a) or 620 nm (b). Taken from DOI: 10.1007/s11120-020-00717-y.

We remark that, although the spectral dynamics of isolated PSI in previous studies resemble our isolated PSI measurement, some differences remain in terms of the clarity and exact energy of the observed features. This would be expected given that PSI protein supercomplexes differ in different photosynthetic organisms (summarised in next paragraph) in terms of their light harvesting components, types of chlorophylls and absorption of different chlorophyll *a* pigments. Furthermore, studies on isolated systems also involve different purification processes (e.g. in membrane or particles, trimer or monomer, whole super complex or core). For these reasons, we compared our whole cell wild-type and mutant spectra to our isolated photosystems spectra (all cyanobacterial systems, and all with the photosystems in their final complex form e.g. we use an isolated PSII dimer, PSI trimers).

In plants and algae, PSI is in a supercomplex of a core and a light-harvesting component (PSI-LHCI). In cyanobacteria, the phycobilisomes (PBS) are mainly an antennae for PSII but also for PSI during state transitions. Plant and algal PSI and associated light harvesting complexes also contain chlorophyll *b*, whereas cyanobacteria only contain chlorophyll *a.* There are other cyanobacterial species that contain far red chlorophylls in their PSII like chlorophyll *d* and *f,*[2] but not in *Synechocystis*. Within the chlorophyll *a* pigments, there are "red forms", which absorb at lower energy than the reaction centre chlorophylls at wavelengths longer than 700 nm (more background and reviewed by Karapetyan et al., 2006 [3]). Red chlorophylls differ in number, position and absorption in PSI in different photosynthetic organisms. The number of red chlorophylls is very species dependent. 5-10% of the chlorophylls are far red in different cyanobacteria.[4] The red Chls in plants are largely located in the peripheral LHCI complexes away from the P700.[5] However, in cyanobacteria these red Chls are located in the core of PSI. In cyanobacteria, the most red forms are located in the edges of the monomers coming together in the PSI trimer.[6] In plants, the red chlorophylls absorb in the 710−730 nm window.[7] In cyanobacteria, the red chlorophylls are more diverse and absorb in the 705-740 nm

region. In *Synechocystis* PSI (our whole cell species), the red chlorophylls absorb in the 707-715 nm window.[8] In Thermoelongatus PSI trimers (our isolated PS species), the red chlorophylls absorb in 708 and 719 nm.[6] *Spirulina platensis* has the most red-shifted chlorophyll known with an absorption centred at 740 nm.[8]

Lastly, we would like to clarify the terminology of the 715 nm feature, ascribed by the reviewer as a bleach. We never refer to this feature as a bleach, as it does not show up in the absorption spectrum and is not present directly after excitation. Rather, the feature at 715 nm corresponds to a stimulated emission from a lower energy state than P680/P700, that is populated within 2 ps after photoexcitation. Critically, the signal rise at 715 nm is inhibited with the addition of DCBQ demonstrating an ultrafast interaction. We use this feature as a visual indicator for early time electron extraction, more properly quantified using global analysis, as a universal decay is difficult to visualise.

Author actions:

- Inclusion of new discussions in **SI Section 1.2** explaining the differences between the controls, and references to wider literature.
- Inclusion of new **SI Section 1.3** comparing our data to previous studies on PSI, with an extensive table of previous works considered in our comparisons.

It would also help if the authors added fsTA spectra at selected times (to the SI) to make it easier to observe the shape and evolution of the TA spectra.

We have now added fsTA spectra for each sample in the SI.

Author actions:

- Added fsTA spectra for each sample (see **SI Figure 8** for PSI, PSII, PSI less cell and PSII less cells, and **SI Figure 13** for cells alone and in the presence of DMSO and DCBQ).

The authors excite at 450 nm, which should initially populate one (or more) higher electronic state(s). Is it possible that some of the fsTA spectra and dynamics are related to higher excited states? It should definitely be possible that excitation and reaction with DCBQ occurs from other states located on other pigments than P680 and P700, which are transiently populated only because of the 450 nm excitation. The results and possible benefits for biohybrid systems would then be limited to a smaller part of the solar spectrum. This must be considered.

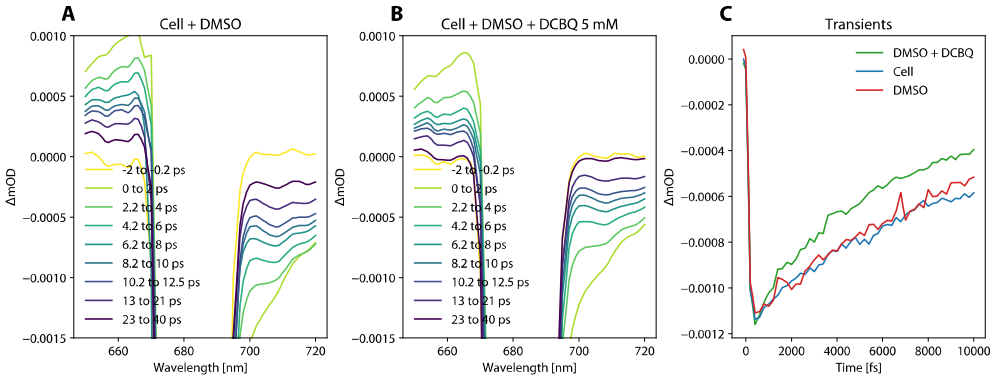
The reviewer raises an important point regarding exciting at 450 nm. At this energy, without a-priori knowledge of the density of states, it is conceivable that some higher excited states interact with DCBQ, which could not be excited by red light resonant with P680/P700 and therefore limit the applicability of the benefits to future biohybrid systems.

In the original submission we provided excitation spectra at 50 nm intervals, (**SI Figure 5**). These results highlighted similar dynamics were observed when exciting over the absorption of PSI and PSII. This suggests we excite, or rapidly cool, to the “ground excited state” of both PSI and PSII when exciting over a broad wavelength band.

To more carefully compare these wavelength tunable results, we repeated our DCBQ cell experiment by further varying the excitation wavelength. Here, we excited P680/P700 directly by using a pump centred at 680 ± 10 nm, where higher excited states cannot be accessed. As we illustrate in Review

Response Figure 2 below, we obtained the same results as with 450 nm excitation, namely an accelerated decay of the low-energy feature at 715 nm. We highlight that the pump scattering prevents us to inspect the bleach kinetics at 680 nm and that this result is in line with previous isolated PSI studies (e.g. Russo et al [1]) which resolved negligible differences between different photo-excitation wavelengths.

Given these observations, we believe higher excited states are not required to explain the results, and that therefore the benefits to biohybrid systems are not limited to a small part of the solar spectrum.



Review Response Figure 2 – **A** Spectra from cells and DMSO excited at 680 nm. **B** Spectra from cells, DMSO and DCBQ excited at 680 nm. The supressed rise at 710 nm is visible. **C** Equivalent data, averaged between 708 and 715 nm, plotted as a function of time, highlighting the DCBQ induced accelerated decay.

Author Actions:

- Carried out new experiments with cells, pumping at the band edge (680 nm), with and without DCBQ. SI Figure 6 added.
- Explained energy dependence in the main text to exclude participation of higher lying states (paragraph 4 of main).

[My main question is whether the manuscript really shows that electrons are extracted by DCBQ, on the time scale investigated.] Finally, on the arguments against energy transfer quenching, there are other possible quenching mechanisms than energy and electron transfer. In addition, Dexter energy transfer does not require spectral overlap (although of course there must be acceptor states at suitable energy). The authors should consider these points.

We attempt to resolve the reviewer's question by laying some foundations to the discussion of quenching, as quenching can mean different things to different fields. We propose that the physical processes available on this timescale must invoke or encompass, in the broadest general terms, either

- Energy transfer
- Electron transfer

If the interaction relies on energy transfer, spectral overlap between the donor and acceptor (here DCBQ) must exist. These may be resolved from absorption and photoluminescence measurements. From our measurements it becomes apparent that DCBQ does not have required accessible states, in

particular spectral overlap for energy transfer. We therefore discount energy transfer in the DCBQ case. We note that Dexter energy transfer also only proceeds if the spectral overlap is non-zero, as encoded through the spectral overlap integral.

If the interaction is based on electron transfer, while it must be short ranged, it does not require spectral overlap, and it would scale with concentration. Our experimental results are consistent with these requirements. The mid-point potential of DCBQ (+0.315 V vs SHE at pH 7) makes it suitable to extract electrons from PSI also (P700*/P700+ = -1.290 V, FB/FB- = -0.590 V) and PSII (P680*/P680+ = -0.660 V) via outer-sphere electron transfer mechanisms. Structural data of PSII and PSI suggests there exist sites in the periphery of the photosystems where mediators may bind sufficiently close to chlorophyll pigments, as understood from Marcus theory, to allow electron transfer.

Among the other mechanisms neither exciplex formation, collisional quenching or static quenching are sufficient to explain our results of an ultrafast accelerated decay in P680/P700. We have tabulated the various mechanisms in a new section (**SI Section 1.3**) which we explicitly refer to in the main text.

Additionally, we stress that we are not basing our conclusions on spectroscopy alone, as we also used electrochemistry as further evidence. We cannot explain the long-observed electrochemistry results (also observed by us, see **SI Section 4**), where the photocurrents enhancements remain upon DCBQ and DCMU addition (**SI Figure 25**). As we know DCMU blocks the Q_B site, it suggests the DCBQ must be extracting electrons before the Q_B site. The protein sites near Q_A has been shown to be accessible to cationic molecules, and not DCBQ. As such, an alternative electron donating site for DCBQ must exist.

Mechanism	Physical Description	Relevance to our System	References
Dynamic Quenching			
FRET	An RC, in its electronic excited state, may transfer energy to an acceptor through nonradiative dipole–dipole coupling.	No overlap between non-reduced DCBQ absorption and PL of RCs. No accessible states.	[9][10][11]
Dexter	Bilateral exchange of electrons.	No overlap between non-reduced DCBQ absorption and PL of RCs. No accessible states.	[12]
Excimer Formation	Short molecule formed from two species, which would not bond if in ground state.	Exciplex formation should be a shallow state, with broad features, which we do not observe. Further, this would not explain current increase upon DCBQ and DCMU addition without the electron transfer mechanism (SI Figure 23).	[13][14]
Static Quenching			
Static Quenching	Ground state complex formed before excitation.	1- Would result in no change in excited state lifetime, contrary to our results 2 – Cell and DCBQ absorption spectrum do not change from cell spectrum.	[15]

Other Mechanisms			
DCBQ Induced Vibrational Relaxation	Changes in structure result in increased thermalisation rate to the ground state.	Invokes the generation of new phonons mediated by DCBQ, unlikely given structure of RCs.	
Diffusion	Excited RC encounters atom that facilitates non-radiative decays	Too slow to act on fs timescale when understood through kinetic theory.	[16]
Electron Transfer	Electron moves from electron donor to electron acceptor.	Short range and requires suitable energetics and binding sites which we have identified.	[13][17]

Referee Response Table 1 – Dynamic and Static Quenching Routes. Quenching routes through triplet state formation have been omitted due to the ultrafast time scale.

Author Actions:

- Added new section in SI (1.5.5) and table that discusses the quenching mechanism in detail, justifying the conclusion that the quenching is due to charge transfer.
- Carried out several attempts to characterise the charge transfer to MV^{2+} (the $MV^{+\bullet}$ absorption) in the ps time scale to provide further evidence of charge transfer. However, the extinction coefficient and population of the reduced species limit observable rise in the red to below the signal to noise limit of our system. Discussion has been added as new **SI section 1.8**.

Then there is a typo in the main text and SI: the overlap requirement for Förster transfer is between the absorption of DCBQ before reduction (not reduced DCBQ) and the fluorescence (not absorption) spectrum of the photosystem.

We thank the reviewer for raising the typos in both the main text and SI. We have reworded to make clear our point regarding these processes cannot be explained by resonance energy transfer.

References

[1] M. Russo *et al.*, "Ultrafast excited-state dynamics in land plants Photosystem I core and whole supercomplex under oxidised electron donor conditions," *Photosynth. Res.*, vol. 144, no. 2, pp. 221–233, May 2020.

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[3] J. H. Golbeck, Ed., *Photosystem I*, vol. 24. Dordrecht: Springer Netherlands, 2006.

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[14] F. Laquai, Y. S. Park, J. J. Kim, and T. Basché, "Excitation Energy Transfer in Organic Materials: From Fundamentals to Optoelectronic Devices," *Macromol. Rapid Commun.*, vol. 30, no. 14, pp. 1203–1231, Jul. 2009.
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[17] R. A. Marcus, "On the Theory of Oxidation-Reduction Reactions Involving Electron Transfer. I," *J. Chem. Phys.*, vol. 24, no. 5, p. 966, Dec. 2004.

Reviewer Reports on the First Revision:

Referee #1 (Remarks to the Author):

The authors have successfully addressed my previous concerns. I have learned a great deal from this paper that I could apply to my own research, so I think they addressed the concern about applicability to practitioners.

Referee #2 (Remarks to the Author):

The authors have provided quite thorough and appropriate responses to the questions raised, and I find them satisfactory. I therefore recommend publication of the manuscript.

----- End of comments -----