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## Functional Architecture of Higher Plant Photosystem II Supercomplexes

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### Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision

17 June 2009

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are enclosed. As you will see, all three referees express significant interest in your work and are broadly in favour of publication, after suitable revision as outlined in the reports. I will not go through all the details here, but I would in particular like to draw your attention to the comments of referee 3 regarding the crystal structure data used as your reference. It would clearly be valuable to use the most accurate structural data available, and I would therefore ask that you revise your model accordingly.

Given the referees' positive recommendations, I would therefore like to invite you to submit a revised version of the manuscript, addressing all the comments of all three reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision. Acceptance of your manuscript will thus depend on the completeness of your responses included in the next, final version of the manuscript.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,  
Editor  
The EMBO Journal

## REFEREE REPORTS

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### Referee #1 (Remarks to the Author):

This manuscript provides important new insights into the structure of the photosystem II-light harvesting complex. The authors have used gentle thylakoid solubilization for isolating photosystem II supercomplexes with different antenna sizes ranging from the core to the large C2S2M2 supercomplex in Arabidopsis. The analysis of these complexes from wild-type and mutant lines deficient in specific antenna proteins by electron microscopy combined with biochemical methods has provided new insights into the hierarchy of assembly of the individual subunits in the supercomplexes. It has also led to several important results such as the identification of a new complex consisting of a monomeric core, a trimeric LHCII (S) and CP26 indicating that the antenna proteins can stably bind to the monomeric core. Moreover a projection map at 12 Å resolution of the C2S2M2 complex has revealed the positions and orientation of the antenna complexes. This has important implications for the main pathways of excitation energy transfer from the antenna to the core. Taken together this work is rather impressive and should be of considerable interest to a large readership interested in the assembly of large macromolecular membrane structures.

### Minor comments

Fig. 2 the positions of CP43 and CP47 should also be indicated. Why are they so fuzzy? . D1 and D2 are not detected because they are too diffuse. This should be indicated in the legend.  
Fig 2 legend: "Note that PsbP is better visible in band 1 and 2 (\*)". The authors mean presumably lanes B1 and B2.

Fig. 3B It is surprising that Lhcb3 is as abundant in B5 (monomeric PSII) than in B4 (Cp24, CP29, M).

Since B8 according to Fig. 1C contains both C2S and C2M, it is surprising that Lhcb3 is absent from this band.

p.6, end of 1st section: replace Figur 3 by Figure 2 (where PsbQ is shown)

### Referee #2 (Remarks to the Author):

The work addresses a central issue in plant photosynthesis, namely the organization of the supercomplexes formed between PSII and its various antennae. It combines biochemical methods with single particle reconstruction to isolate and characterize homogeneous populations of such complexes. The results provide new insights into the interactions and spatial relationships between the different subunits that constitute PSII-antenna assemblies and have important implications on energy transfer, non-photochemical quenching, and photo-inhibition. Overall, it's a well thought out and performed work and I tend to agree with most of the interpretations.

### Comments:

1. Given its importance to the manuscript, the authors should describe in the Results section how their isolation procedure, which allows obtaining homogeneous complexes, differs from previous ones.
2. In the legend of Fig. 2 the authors note that the band corresponding to PsbP is "better visible" in lanes B1 and B2. However, this is not the case for lane B2. In general, I think that assignment of the bands corresponding to Lhcb1,2,3, PsbP and PsbS in this figure is somewhat problematic, as they are not well separated.
3. In Fig. 3B, it is not clear why a strong Lhcb3 signal is observed in lane B5, which represents the monomeric PSII core, and is absent in lane B8, which should contain Lhcb3 (in C2M).
4. The rationale for performing the experiment shown in Fig. 5 should be explained in the Results,

in which it is not referred to at all.

5. The models proposed by Betterle et al. and Kiss et al. (p. 15) suggest a different mechanism for PsbS function. I am not an expert in the field, but it seems to me that the results derived by the authors are not inconsistent with the model proposed by Betterle et al., as noted by the authors.

6. Given the resolution obtained for the C2S2M2 supercomplex, I would have toned down the parts concerning the implications regarding energy transfer pathways (e.g. "A projection map...reveals...the main pathways of excitation energy..." p. 17).

Referee #3 (Remarks to the Author):

This study represents significant progress in the analysis of the pigment-protein photosystem II supercomplex from higher plants. It is a valuable study. However, there is one major aspect in which the analysis is not up to date. In the Introduction to the structure (pp. 1-2) and in the reconstruction of a 3D atomic model of the PSII-LHCII supercomplex (p. 10), the crystal structure of the cyanobacterial PSII reaction center of Loll et al, 2005 was used as the reference structure. The refinement of this structure and its effective resolution have now been significantly improved, and a significant number of additional bound pigment molecules resolved, among the structure differences, in the study of Guskov et al., 2009. It is necessary to consult these latest data to update the Introduction and to check and probably to update details of the 3D atomic model of the supercomplex.

1st Revision - authors' response

24 June 2009

Thank you very much for your letter. We are glad that all referees think that our work represents a considerable step forward in the understanding of Photosystem II. We have changed the manuscript according to their comments and as suggested by referee 3 we have now used the latest PSII-core structure for our modelling. We thank the referees for their help in improving our manuscript.

Referee #1 (Remarks to the Author):

*This manuscript provides important new insights into the structure of the photosystem II-light harvesting complex. The authors have used gentle thylakoid solubilization for isolating photosystem II supercomplexes with different antenna sizes ranging from the core to the large C2S2M2 supercomplex in Arabidopsis. The analysis of these complexes from wild-type and mutant lines deficient in specific antenna proteins by electron microscopy combined with biochemical methods has provided new insights into the hierarchy of assembly of the individual subunits in the supercomplexes. It has also led to several important results such as the identification of a new complex consisting of a monomeric core, a trimeric LHCII (S) and CP26 indicating that the antenna proteins can stably bind to the monomeric core. Moreover a projection map at 12 Å resolution of the C2S2M2 complex has revealed the positions and orientation of the antenna complexes. This has important implications for the main pathways of excitation energy transfer from the antenna to the core. Taken together this work is rather impressive and should be of considerable interest to a large readership interested in the assembly of large macromolecular membrane structures.*

*Minor comments*

*Fig. 2 the positions of CP43 and CP47 should also be indicated. Why are they so fuzzy? D1 and D2 are not detected because they are too diffuse. This should be indicated in the legend.*

We have now added this sentence in the legend of figure 2: "D1 and D2 are not visible in this gel system because they maintain a partial folding during migration which makes them appearing diffused". The fuzzy appearance of CP43 and CP47 in the gel is also due to the fact that they are not fully denaturated. We have however decided to use this gel system because it gives the best results for the region that contains most of the proteins that are interesting for this work such as the antenna complexes and OEC subunits. We have now indicated in the gel the position of CP47 and CP43, as suggested by the referee.

*Fig 2 legend: "Note that PsbP is better visible in band 1 and 2 (\*)". The authors mean presumably lanes B1 and B2.*

Yes, thanks. We have changed the sentence in the legend which is now: "Note that PsbP is better visible in lines B1 and B2."

*Fig. 3B It is surprising that Lhcb3 is as abundant in B5 (monomeric PSII) than in B4 (Cp24, CP29, M).*

*Since B8 according to Fig. 1C contains both C2S and C2M, it is surprising that Lhcb3 is absent from this band.*

Lhcb3 is less abundant in B5 than in B4 as can be seen in figure 2. In the gel in figure 3 the gel line of the B4 band is broader than that of B5 thus giving similar intensity in the blotting, although the area of the bands is different. Moreover band B5 is highly contaminated by B4 especially because using this very mild solubilisation the amount of monomeric core is very small. We added this sentence in the legend of figure 3: "Band B5 was highly contaminated by B4 also due to the low amount of PSII core present in this preparation"

Yes, B8 contains C2M, but the amount of these particles is around 5%, while 95% is composed by C2S particle. We have now changed the sentence (page 5): "B8 (contains) mainly C2S and some C2M supercomplexes" into "B8 (contains) C2S and very few C2M supercomplexes (around 5%)"; and we have added on page 6 "...B8, note that this last band contains only a very few C2M particles"

*p.6, end of 1st {section sign}: replace Figur 3 by Figure 2 (where PsbQ is shown)*

Thanks. We have now corrected this in the text

*Referee #2 (Remarks to the Author):*

*The work addresses a central issue in plant photosynthesis, namely the organization of the supercomplexes formed between PSII and its various antennae It combines biochemical methods with single particle reconstruction to isolate and characterize homogeneous populations of such complexes. The results provide new insights into the interactions and spatial relationships between the different subunits that constitute PSII-antenna assemblies and have important implications on energy transfer, non-photochemical quenching, and photo-inhibition. Overall, it's a well thought out and performed work and I tend to agree with most of the interpretations.*

*Comments:*

*1. Given its importance to the manuscript, the authors should describe in the Results section how their isolation procedure, which allows obtaining homogeneous complexes, differs from previous ones.*

We have now added this sentence on page 4: "To this aim the membranes were solubilized with a very low concentration of -DM and the different complexes were separated on a dense sucrose gradient which also contained a very low concentration of detergent to avoid destabilization of the complexes during ultracentrifugation. The detergent concentration was about 4-8 times less than what is normally used for a sucrose gradient, but still sufficient to maintain the complexes in solution. All the different steps of the procedure were performed in dim light and in the cold. This is very important since the temperature has a large effect on the stability of the complexes

(Supplementary Figure S1).

*2. In the legend of Fig. 2 the authors note that the band corresponding to PsbP is "better visible" in lanes B1 and B2. However, this is not the case for lane B2. In general, I think that assignment of the bands corresponding to Lhcb1,2,3, PsbP and PsbS in this figure is somewhat problematic, as they are not well separated.*

We agree with the referee that the assignment is not easy because Lhcb1 and Lhcb2 overlap as well as Lhcb3 and PsbS. However, this is not the case for PsbP, which is separated from the other complexes. To check the assignment we have done western blotting analysis using antibodies against Lhcb1,2,3, PsbS and PsbP (Fig. 3 and Fig. S2) and we have also analysed the npq4 mutant which does not contain PsbS and thus allow to resolve Lhcb3. All the data are totally consisted with the assignment presented in fig. 2.

*3. In Fig. 3B, it is not clear why a strong Lhcb3 signal is observed in lane B5, which represents the monomeric PSII core, and is absent in lane B8, which should contain Lhcb3 (in C2M).*

Indeed B8 contains C2M, but the amount of these particles is around 5%, while 95% is composed by the C2S particle. We have now changed the sentence (page 5): "B8 (contains), mainly C2S and some C2M supercomplexes" into "B8 (contains) C2S and very few C2M supercomplexes (around 5%)";

B5 is contaminated by B4 because our gradient is optimized to separate high molecular weight complexes, but not the low molecular weight complexes, and also because in this preparation the amount PSII monomeric core is very small, as it is now written in the legend of fig. 3.

*4. The rationale for performing the experiment shown in Fig. 5 should be explained in the Results, in which it is not referred to at all.*

We have now added the following sentence on page 8: "In order to determine the role of the individual antennas in the architecture and in the stability of the Photosystem II supercomplexes, we have used the solubilization procedure described before for the WT on grana membranes prepared from three knock-out (KO) lines lacking Lhcb3, CP24 or CP26."

*5. The models proposed by Betterle et al. and Kiss et al. (p. 15) suggest a different mechanism for PsbS function. I am not an expert in the field, but it seems to me that the results derived by the authors are not inconsistent with the model proposed by Betterle et al., as noted by the authors.*

We agree with the referee, indeed this part of the discussion was not clearly written. Our point was that in vitro we do not see any modification of the organization and thus that something in addition to the low luminal pH is needed to explain the in vivo results of Betterle et al.. We have now rephrased this part:

We have REPLACED the paragraph: "This proposal is not in agreement ..might be necessary for the activation of PsbS." (page 15-16) BY THIS ONE : "Our results show that the solubilization has an identical effect on WT and npq4 mutant (Figure 5A), also at low pH (Figure 5B) when PsbS is protonated and should facilitate the detaching of LHCII from the core in vivo (Betterle et al., 2009; Kiss et al., 2008). Our results thus suggest that the protonation of the two luminal glutamate residues (Li et al., 2004) is not sufficient to activate PsbS and regulate the interactions LHCII-core. Other factors such as a particular ion or the presence of a pH (and not just a low pH as in the in vitro experiment) might be necessary for the activation of PsbS"

*6. Given the resolution obtained for the C2S2M2 supercomplex, I would have toned down the parts concerning the implications regarding energy transfer pathways (e.g. "A projection map...reveals...the main pathways of excitation energy..." p. 17).*

We have changed the sentence on page 17 " A projection map " into "A projection map at 12 Å resolution of the C2S2M2 supercomplex reveals the positions and the orientations of the antenna complexes and allows to suggest the main pathways of excitation energy transfer from the antenna to the core"

*Referee #3 (Remarks to the Author):*

*This study represents significant progress in the analysis of the pigment-protein photosystem II supercomplex from higher plants. It is a valuable study. However, there is one major aspect in which the analysis is not up to date. In the Introduction to the structure (pp. 1-2) and in the reconstruction of a 3D atomic model of the PSII-LHCII supercomplex (p. 10), the crystal structure of the cyanobacterial PSII reaction center of Loll et al, 2005 was used as the reference structure. The refinement of this structure and its effective resolution have now been significantly improved, and a significant number of additional bound pigment molecules resolved, among the structure differences, in the study of Guskov et al., 2009. It is necessary to consult these latest data to update the Introduction and to check and probably to update details of the 3D atomic model of the supercomplex.*

We have re-done our modelling using the new structure both in figure 4 and in figure 7. We have also changed the references as suggested by the referee.

2nd Editorial Decision

15 July 2009

Many thanks for submitting the revised version of your manuscript EMBOJ-2009- 71367. It has now been seen again by referee 3, who now supports publication but still has a couple of outstanding issues (detailed below in his/her report) that need to be incorporated into a revised version of the text before we can accept the manuscript.

I look forward to receiving your revision.

With best wishes,

Editor  
The EMBO Journal

Referee 3 comments:

1. Although the improved refinement of Guskov et al. has now been used for reconstruction, there is no indication in the text, the supplementary material, or the rebuttal letter regarding the quality of the fit. The utilization of the reference structure would be more meaningful if such information was provided.
2. Discussion, p. 10. The summary of the atomic resolution structures of electron transport complexes has omitted the structure of the PSI complex (Jordan et al., Nature, 2001). For the b6f complex, a native structure with improved resolution is provided in Yamashita et al., J. Mol. Biol., 2007.

2nd Revision - authors' response

15 July 2009

Thank you for your letter. We have now modified the manuscript according to the last remarks of reviewer 3.

Below the modifications of the text suggested by the reviewer are listed.

*1. Although the improved refinement of Guskov et al. has now been used for reconstruction, there is no indication in the text, the supplementary material, or the rebuttal letter regarding the quality of the fit. The utilization of the reference structure would be more meaningful if such information was provided.*

There are no standard criteria for fitting 3D X-ray data into 2D EM maps, like it is for 3D EM data

(see Fabiola et al. Structure 2005 vol. 13 page 389). But in general, the precision of fitting is considered to be about 2-3x the resolution. It would mean that this would be around 5 Angstrom. We added a sentence on this in the text.

Page 8. "Although no standard criteria are available for fitting X-ray data into 2D EM maps, the resulting pseudo-atomic data can be considered to have a precision extending the resolution of the EM data at 12 Å, in the range of the size of an alpha helix or a chlorophyll molecule."

We also modified a sentence in the discussion to stress the improvement of the new structure.

Page 11. "This allowed the reconstruction of a 3D pseudo-atomic model of the full PSII-LHCII supercomplex using the crystal structures of the LHCII trimer (Liu et al., 2004) and the recent refined structure of the cyanobacteria core (Guskov et al., 2009) which presents the full assignment of the small PSII subunits and an improved localization of the cofactors".

*2. Discussion, p. 10. The summary of the atomic resolution structures of electron transport complexes has omitted the structure of the PSI complex (Jordan et al., Nature, 2001). For the b6f complex, a native structure with improved resolution is provided in Yamashita et al., J. Mol. Biol., 2007.*

We have now added the references suggested by the reviewer.