Reviewer's opinion on the dissertation entitled „On the role of light harvesting complex II in regulating the excitation energy flow" by Petar H. Lambrev

The topic chosen by the Applicant is undisputably very important. One of the admirable beauties of the photosynthetic system is its efficiency accompanied by its ability to withstand higher than needed irradiation intensities without significant deterioration – we would like to learn as much as possible on how this is achieved. In this respect light harvesting complex II is one of the key entities, and the energy flow within and from this complex crucially influences both aspects of photosynthesis mentioned above.

Photosynthesis research is an enormously wide field, pursued by a vast number of researchers. It is only natural, that important results can mostly be obtained with very advanced experimental techniques. The work covered by the dissertation shows clearly, that with the help of intense international cooperation Hungary is a key player, and the experimental techniques available at the Biological Research Center in Szeged places the Institute among the leading laboratories.

The dissertation contains 134 pages (text and figures) and nearly 300 literature references. The format of the latter conforms to standards used in photobiology, namely in alphabetical order of the first author, which, in some cases, leaves room for ambiguity (e.g. there are three papers referred to as Akhtar *et. al.* (2019)). The dissertation is easily readable, and it contains very few typing mistakes – barely enough for the opponent to show, that he indeed has read the whole dissertation carefully (an amusing one is the "law-salt buffer" on age 77). The English is clearly understandable. The list of abbreviations is very helpful, even though it is not complete (e.g. CsmA on page 20 and PsbS on page 42 is not explained).

One of the strength of the dissertation is the chapter on the dynamics of energy transfer within LHCII. It confirms that energy equilibration within LHCII occurs on extremely short timescales, utilizing both downhill and uphill energy transfer. At the same time it is a good example how advanced experimental technique (in this case by using only 1 nJ pulses for excitation) can get rid of the misleading side effects of singlet-singlet annihilation. These experiments enabled new structure-based modelling to gain a more accurate and detailed understanding of the light harvesting dynamics. Not only a phenomenological model has been elaborated for EET at liquid nitrogen temperatures, but a successful attempt has been achieved for mapping exciton states to individual (or pools of) Chls in LHCII.

Remarks and questions:

Page 9, Fig. 2.1.: there are two C atoms denoted as No. 13., and in the legend there is an obvious typing mistake showing a methyl group as -CHO.

The chapter Background is a very instructive summary of both the basics of light harvesting, including macroorganisation of thylakoid membranes, and of the spectroscopic methods used, detailing CD and ACD spectroscopy as well as two-dimensional electronic spectroscopy. Photosynthesis research is a very large field within photobiology, so such a good introduction is very valuable for those not familiar with the specific features of the field.

While reading this chapter two questions occurred to me:
1. Fig. 2.2. shows the Perrin-Jablonski diagram of Chl. In the sister field of porphyrin research fluorescence from the upper excited state S_2 has been observed – has such an emission for Chls been detected?
2. The orientation of samples in anisotropic CD measurements is crucial. Even though larger structures (like thylakoid membranes) are more or less easy to align macroscopically, is it necessary

to include some kind of averaging to account for the imperfect alignment? On page 79 it is stated that ACD spectra were independent on the method of orientation – since neither of the two methods of orientation (gel compression and dehydrated film) is likely to result in perfect orientation, and the spectra have a lot of characteristic features (peaks and valleys), it would have been better to show, how much the spectra are similar for the two orientation methods.

Sample preparations is an important part of experimental techniques, due to the specificity of the nature of LHC II. Washing out the detergent without allowing protein aggregation has been checked with control experiments, where the washed gels were measured after incubation with detergent-containing buffer. I am not totally convinced, that such a control proves that the detergent has been washed out completely – please explain your line of reasoning.

How much sample deterioration has been observed during the synchrotron radiation CD spectroscopy experiments at the Diamond beam line?

The experimental technique used to measure fluorescence spectra at low temperature is intriguing. It is stated that the sample contains approximately 0,5 μg Chl.cm^{-1}. Is this a typing mistake, should it read cm^{-2}? How was the formation of condensation on the cuvette faces avoided at liquid nitrogen temperature in the confined space of the fluorimeter sample chamber?

Please explain the phrase "closing the RC by applying a blue light pulse" (top of page 52.). In the same experimental setup continuous actinic light is employed for the induction of NPQ - does this light not disturb the measurement of Chl fluorescence in the red region?

In time resolved fluorescence measurements the instrumental response function was measured using 0,2% milk as scattering medium. What is the advantage of using milk instead of silica? The natural ingredients of milk might give rise to autofluorescence – how can this be ruled out?

In 2D spectroscopic measurements an optomechanical chopper was used to "detect and correct for" scattered light. Please explain how comes a mechanical device into play on the timescale used in 2D spectroscopy.

Discussion of global lifetime analysis and the use of average lifetimes is described too late (on page 85 – Fig. 5.8. on page 63 shows average lifetimes much earlier). As a matter of fact I do not see any advantage in using average lifetimes, when all the parameters of the multiexponential fitting are known. Global analysis is a very versatile method, however wasn't it used in a too-much strained manner on page 88? It is stated that three lifetimes – 0,54 ps, 4,7 ps and 3,2 ns – were necessary to obtain a good fit in the time window of 0,15 to 60 ps; parameter estimation so much outside of the experimental time window must contain a very high uncertainty.

How much does the excitation spectral width (~15 nm) affect the evaluation of downhill/uphill EET (page 88)?

Singlet-singlet exciton annihilation is mentioned several times (e.g. page 91) – what is known about this interaction? What are the products, where does the excitation energy dissipate?

The absorption features of Chl *a* and *b* are not widely different - are the changes in the Chl *a* and Chl *b* ratio in Bryopsis corticulans responsible for the increased blue-green absorption in this syphonous alga? Isn't the change in the carotenoid composition more important? How does this relate to the mechanism of photoprotection, why is this alga a good model to study photoprotection?

The functional domain size determination using time resolved fluorescence spectroscopy is very interesting. Unfortunately neither in the dissertation, nor in the original paper is mentioned, which of the three isomer dinitrobenzene has been employed as quencher. I have to presume that within the photosynthetic community quenching of Chl fluorescence by PPQ or DNB are well know – as an outsider, I would be very much interested how these compounds quench fluorescence. In the original publication it is well explained, why the measured quenching formally resembles dynamic quenching (this is not even mentioned in the dissertation) – however such a formal resemblance does not justify the publication of bimolecular quenching rate constants in the 10^{13} and 10^{14} $M^{-1}s^{-1}$ range. Such high values are meaningless, impossible for bimolecular reactions, only their relative magnitude may bear importance. I would appreciate an explanation on why and how the same quencher is more effective, when quenching LHC II aggregates as compared to solubilized trimers (the enhanced activity is even more surprising, since the fluorescence lifetime of aggregates is considerably shorter, than that of the solubilized ones).

The data shown in Fig. 6. 25. are not understandable. It is claimed that panel A shows absorption spectra, while panel B shows the difference of this two spectra together with LHCII only membranes (illustrating, that the difference in panel A is due to LHC II). However the scale in panel A is between 0 and 1,5, it is surprising that the difference spectrum has the same range. While this might be the result of normalizing the spectra, there is a disturbing fact *i.e*. in panel A the difference between the spectra is larger just above 450 nm, as compared to that just below 450 nm, while the difference spectrum in panel B is more intense below 450 nm, which cannot be due to any normalization. Please explain.

In Fig. 6.28. panel B three spectra are shown. Isn't it possible that the three spectra are almost identical (a normalization would easily answer this)?

The review would not be complete without stating the opinion of the reviewer on the thesis (in the dissertation called "Summary) of the work. The first one states that the structure and excitation properties of LHCII are sensitive to the molecular environment of the complex. While I fully agree with this statement, this is a triviality – the absorption and excited state characteristics of almost all naturally occuring complex systems are indeed very sensitive to the molecular environment. Nevertheless, points a. and b. nicely summarize the findings of the work. I accept all 7 points as new scientific results, noting that ACD spectroscopy seems to me a more-or-less qualitative tool (as compared to absorption spectroscopy), which is natural for an emerging new technique. In my opinion items 2, 4 and 6 are the most valuable contribution.

In summary, I suggest that the Doctoral Council should advance the procedure for the title of "Doctor of the Academy" by appointing a date for the defence procedure, and after a successful defence grant the title to the Applicant.

Budapest, 29th November 2022.

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