REVIEW OF THE DISSERTATION OF PETAR LAMBREV, PRESENTED FOR THE DOCTOR OF ACADEMY TITLE

The significance of the research subject

I fully agree that the theoretical details of light harvesting, and the correlations between structures and the function of the pigment systems must be clarified in detail. An obvious contradiction is spreading between basic and applied research fields nowadays: Plenty of applied research scientists are satisfied with simple chlorophyll (Chl) content data often measured indirectly with SPAD and similar "chlorophyll meters" deeming that the increase of the Chl content automatically means a higher photosynthetic activity.

I understand and recognize the Candidates' research strategy i.e. using isolated particles and model systems. The LHCII subunits contain plenty of chromophore molecules, and the distances between them allow various exciton interactions of their π-electron clouds. In addition, the geometrical factors and the interactions of the lipids and proteins of the thylakoid membranes should be considered. To study such complex systems the application of various models are suitable. The properly interpreted models show real possibilities to understand complex correlations between the native structures and functions.

The structure of the dissertation

The whole dissertation is carefully elaborated, however, its structure differs from the traditions in many aspects and I found several text edition problems and mistypes.

The first chapter is the "**Introduction**" which starts with general and basic information pieces.

The next main chapter has the title "**Background**". I expected here a literature review like in usual scientific publications. However, also this chapter starts with basic information (for example the summary of photosynthetic pigments) then deals with detailed descriptions of the spectroscopic techniques used in this project, and returns again to basic information pieces about the photosynthetic pigment-protein complexes and to the "Architecture of the thylakoid membrane". The literature review details are specified in the description of results which are itemized in the main chapters 5. and 6. "**Structural and functional plasticity of LHCII**" and "**Dynamics of energy transfer**", i.e. we cannot find a separate "Results" chapter. The description of the results are in sub-chapters of these which contain literature reviews connected to the given results and also the discussion and/or interpretation. Considering the complexity of the whole project, I can understand the Candidates' motivation to choose this form but it was sometimes a challenge to recognize the published results of the Candidate. Unfortunately, the references do not help everywhere; there are long text details starting or ending with only a few references despite the dissertation containing 380 references. Since the Candidate is not the first author in all 16 papers giving the basis of the dissertation, we can read different names in citations in which the Candidate was the third or last author. It would be easier to find the Candidates' publications if the self-citations were differently printed, for example in bold. The citations to the publications are often missing from the **figure legends** which needed further detailed search. Despite these difficulties, **I do declare here, that I found all results of the dissertation in the 16 publications of the Candidate listed in the thesis booklet.**

The **Aims** of the project are summarized in 4 points however, the "**Summary**" chapter contains 7 main points with 14 subsections.

We can find a 7.5-page summary of the **Materials and Methods**. It is understandable that this chapter contains only partial descriptions and cannot be detailed in the same way as in the 16 publications of the Candidate. However, I found overlaps between the descriptions of the spectroscopic methods and the information given in the "Background". Suitable references were satisfactory here.

As mentioned above, the results of the Candidates' research projects are grouped into chapters: No. 5 entitled "Structural and functional plasticity of LHCII" and No. 6. "Dynamics of energy transfer". These main chapters contain 10 and 8 sub-subsections, respectively, which are good summaries of the results published by the Candidate. I won't comment on all of the 18 items, instead, I raise questions, and comments only on experiments or results which I found unclear.

Comments and questions about the research materials containing detergents

In the Materials and Methods, we can find that the Candidate used α-DDM and ß-DDM detergents for the isolation of LHCII: 0.7 % α-DDM or ß-DDM and 15 min incubation on ice was used at extraction and then, after centrifugation the supernatant was transferred into a 0.06 % detergent containing 5mM Tricine buffer and 0.4 M sucrose. (Page 48) Among the results, 0.03 % detergent concentration is written in the 5.2 figure legend, 0.1 % ß-DDM in 5.3 figure legend. **What was the reason for using various detergent concentrations?**

According to *Protein Science (1994) 3:1975-1983*, and *Methods in Enzymology Volume 182, 1990, Pages 239-253,* the critical micellar concentration of DDM is **0.01 %** in water at room temperature. The great concentration values during isolation and then their dilution brings up the possibility of preparing first reversed micellar systems (with hydrophilic phase inside) and then phase transition into usual (with hydrophilic phase outside) micellar systems at dilution. **In which phase are the LHCII units localized? Can we get information about the structures of these colloid systems? Are there results in this research field about the DDM concentration dependence of the LHCII solubilization and the parallel changes of the spectral properties? What are the particle sizes in the samples with various DDM concentrations; what is the DDM micelle aggregation number?**

Comments on the appearance of the (-) 491 nm CD band

These experiments are logically planned and the interpretations are correct: Varying the components of the different systems, the (-) 491 nm CD band was proven as an effect of interactions of LHCII units with detergent or lipid structures. I fully agree with the Candidates' comment that the detergent micelles are only flawed representations of the native state and the model systems must be interpreted properly. **Is it possible to identify the electronic transition corresponding to this signal?**

I found in chapter 5.1. variability of the CD band ratios in spectra of the aggregates shown in figures 5.1B, 5.2B. **What is the reason for these band amplitude variations?**

Comments on the Far-red-emitting states associated with quenching: 5.2.2.

These measurements were done with samples frozen at 77 K. At this temperature, the molecular environment and the CT distances, therefore the energy migration efficiency and the

vibrational freedom of the complexes differ from those at 293 K. **What is the opinion of the Candidate, how can we interpret these results on TM-s in their physiological states?**
I could not find enough explanation to the Figure 5.10. (page 65.). In panel A, curves are shown in different colours, without the meaning of these colours, we can find out that they correspond to the colours of block frames in panel B. An explanation of the figure labels is missing. In the cited paper (Ostroumov et al. 2020) I found data about measurements with samples cooled to 5, 30, 50, 100 and 170 K in the referred publication but no data with 77 K. **Why were the 77 K results presented here, where were these results (shown in Figure 5.10.) published?**

The chapter on the two-dimensional electronic spectroscopy results is significant and it is an attractive part of the dissertation. This method and the analyses of the results provide us with important details of the energy distribution within the LHCII trimers and aggregates.
A very important part of the dissertation is the comparison of the results measured on LHCII trimers to those of LHCII aggregates. **It would be interesting to know the aggregation number of the studied LHCII macrodomains.**

The native thylakoid membrane components must influence the charge distribution in the whole photosynthetic unit. **Are or can be the two-dimensional spectroscopy measurements extended to the thylakoid membrane units?**

Comments to the chapter "Summary"

I expected a list of conclusions showing the results that the Candidate considers as the main conclusions of his scientific work. Instead, a 3.5-page long description is here, containing 7 titles printed in bold (and a little shorter text with inserted figures is in the thesis booklet).
My opinion is, that not all of these emphasized prints are specific. I won't repeat these titles, instead, I summarize my opinion about the contents of the chapters. I consider new scientific results the statements printed in bold and italic in the next 7 points:

1, This paragraph states first the importance of the molecular surroundings of the LHCII complexes. To my knowledge, this is basic and general information in biochemistry.

My opinion is that the main result of this subject is that the Candidate could create different models which have native absorption and fluorescence properties but their structures can be different. However, the structural and functional plasticity of the complex needs to be considered when inferring physiological function from in vitro experiments. This statement is important because both, the DDM micellar solutions, the proteoliposomes, and the reconstituted membranes are generally used in projects studying the LHCII complexes.

2, ***The Candidate experimentally proved that the LCHII complexes may have distinct charge transfer states at excitation which is important in the self-regulation of light harvesting and in the directional energy migration. With sophisticated spectroscopy methods, fluorescence lifetimes, and kinetic components of non-photochemical quenching were analyzed.***

3, ***Structure-based exciton models were identified and validated; the experimental results were compared with calculated (predicted) ACD spectra.***

4, ***Important details of the energy transfer between the Chl-s were identified, which contributed to creating the structural model.***

5, Further and theoretically important details of the LHCII properties were given in works with the alga *Bryopsis corticulans* having special pigment composition which proves that ***the LHCII is a spectrally tunable light-harvesting antenna.***

6, ***Determined the maximal aggregation number of LHCII trimeric subunits to be limited to about 25***, fitting to the general idea about the "photosynthetic unit".

7, ***The delivery of the excitation energy from LHCII directly to PSI was shown in model membranes which may not have the native structure of thylakoids.*** This result can be important in constructing efficient artificial structures but its inference to the general plant physiology is uncertain.

Minor comments

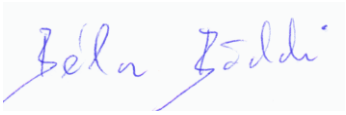
- The **Title** seems to show a kind of uncertainty. Why "On the role" is used to formulate it? A direct statement would be more expressive.
- The last paragraph of the Introduction is a description of the "Table of contents", it is redundancy.
- Page.9. : The subscription of Figure 2.1 contains a mistake: "the methyl group (-CHO) at position C7 is replaced by a formyl group (-CHO)". The figure is correct, but in the legend, a real methyl ($–CH_3$) group is needed.
- Page, Q, and B absorption maxima of Chl-a dissolved in acetone are indicated. It's very difficult to remove water from acetone thus in addition to the Chl-a-acetone monosolvates different Chl-water species are present. The details of the spectral properties for Chls were studied in a water-free diethyl ether solution which contains exclusively mono-solvate Chl-a-ether molecules (see for example Houssier, C. and Sauer, K. BBA 172, 476-491 (1969), BBA 172**,** 492-502 (1969), J. Am. Chem. Soc. 92, 779-791 (1970)).
- Page 58..: Figure 5.3 B. The figure legend contains a mistype: The difference spectrum "unsolubilized minus solubilized" must be "solubilized minus unsolubilized".
- Page 60.: In Figure 5.5. Only one Y-axis is shown but the CD spectra are shifted along this axis, i.e. each spectrum has its own 0-value. The Y values between -0.5 and 2 have no meaning.
- What is the reason of the few nm differences (shifts) in the spectra? Are all spectra shown in figures means of several recordings or are the band position differences due to baseline correction difficulties or data collection frequency problems? (My experience is that to calculate the difference spectrum, at least 0.1 nm data collection frequency is needed.)
- Some of the results are presented on wave number basis in the original publications, however, the dissertation shows spectra and calculations in wavelength function. What is the reason for this conversion?

The above-described comments and questions do not query the outstanding scientific value of the results summarized in this dissertation.
The scientific activity of the Candidate, his results, and his contribution to the photosynthesis research field are outstanding on the international level. He worked in cooperation with internationally recognized leaders in this field and worked in modern, well-equipped laboratories. The whole project was supported by internationally recognized grants. His publications (listed separately only in the thesis booklet) were published in high-quality

journals. I consider all of the results of these 16 publications and thus the results presented in this thesis, original and genuine.

My firm belief is that the scientific work of Petar Lambrev fulfills the requirements of the doctoral title of the Hungarian Academy of Sciences. I consider the results presented in this thesis suitable for open discussion, and in case of a successful defense, I recommend the Committee award the Doctor of Academy degree.

Budapest, 03. 11. 2022.



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