Answers to Prof. Péter Maróti

I sincerely thank Prof. Maróti for the extraordinarily detailed dissection of my work, for providing insightful critique, and identifying problems and questions that will shape my future work.

1. Structure of the LHCII

1) *While the common feature of natural light-harnessing systems in photosynthetic bacteria (LHI and LHII) is a ring of any order of magnitude, in plants the organisation is mainly trimeric (or multiples thereof).* ***Do you have a reasonable explanation*** *for that?*

   This is an interesting question; unfortunately, I do not know the answer with certainty. My personal view is that there are two aspects to this question – evolutionary and biochemical. Biochemically, the bacterial antenna is composed of smaller and simpler polypeptides that form fewer contacts and naturally associate into rings, while the plant complexes having multiple transmembrane helices that expose a more complex interaction surface. That said, oxygenic phototrophs can also form rings of polytopic transmembrane light-harvesting complexes, as is the case of Pcb and IsiA rings around Photosystem I in cyanobacteria. Evolutionary, one could hypothesize that the plant light-harvesting complexes are optimized to harvest light at different incident angles while simultaneously packing pigments more densely.

2) *Theses 1 and 2 include the dependence of the structure and mechanism of LHCII on molecular surroundings (environment) concluded mainly from measurements carried out in different model systems where the lipid/protein ratio was one of the essential variables. An important but obvious question arises whether the (rather general and still indirect)* ***declarations can be made more specific to lipids in the complex****?*

   No. The lipids in LHCII, namely phosphatidyl glycerol, are required for trimerization and binding the lowest-energy chlorophylls. Extracting or replacing the phospholipid results in monomerization and spectral blue-shift, however, those are not the changes that I refer to in the dissertation.

3) *It is well known that the high-resolution structure of plant C2S2M2-type PSII—LHCII supercomplex reveals remarkable structural roles of lipid molecules in stabilizing the P511 core complex (Sheng et al.2018). Moreover, they contribute to oligomerization of PSII core dimer and LHCII trimers and mediate the assembly between PSII and the peripheral antenna complexes including LHCII, CP29, CP26, and CP24. Additionally, they might influence the biological function of the supercomplex by interacting with the neighbouring protein subunits and the function-related cofactors (e.g., chlorophylls and carotenoids). The interesting problem remains open to me:* ***how the interfacial lipid molecules affect the energy transfer, the electron transport kinetics, and the spectroscopic features of the PSII—LHCII supercomplex****?*

   I agree that this is an interesting problem and one of fundamental importance to understanding the still enigmatic role of specific lipids in photosynthesis. Prof. Maróti is right to identify this as a major question, one that will guide my research in future. The work on LHCII alone evidently cannot answer it, however, we have recently found that the spectroscopic features of the Photosystem II core complex also depend on the lipid

environment, although in a rather different manner and probably for different reasons than in LHCII.

2. Non-photochemical quenching

4) *Recent results from Bielczynski et al. 2022 indicate that qE rise is not accompanied by a structural disassembly of the PSII supercomplexes. Based on your thesis 6 and 7 and on the general discussion of the dissertation, a firm statement about the structural changes in the PSII upon high light excitation can be deduced.* ***How do you comment the results which seem (in the published form) to contradict this view?***

   The mechanisms of NPQ are infamously contradictory and have been polarizing the research community for decades. In my opinion this situation is due to the simultaneous occurrence of numerous independent but complex mechanisms that result in NPQ. That said, there is consensus on several points, and one is that structural changes at the membrane level are associated with NPQ. The results of Bielczynski et al. are not necessarily in contradiction with our spectroscopic data and interpretation. They show that short illumination of thylakoids does not lead to disassembly of the C2S2M2 supercomplexes but still observe the appearance of an additional band of oligomeric LHCII. They attribute this to additional trimers disconnecting from PSII, which is the cornerstone of the aggregation model of NPQ.

5) *The nonphotochemical quenching (NPQ) protects photosynthetic organisms from excess irradiation. This process involves the mechanism where the excess excitation energy is dissipated through heat that converts to mechanical vibrations. This is just the subject of the photoacoustic spectroscopy, that was not mentioned in the introduction of the dissertation. The photoacoustics has gained broad fields of applications due to the extreme sensitivity and compactness of the method. What do you think,* ***why this method has not been used to study NPQ?***

   This is an interesting suggestion. Several groups have tried using photoacoustic to quantify NPQ (Havaux, Malkin, Lannoye) but I do not know of any recent publications on the topic. I would agree that the approach deserves revisiting using newer instrumentation. We are currently setting up a cooperation with the University of Szeged to perform photoacoustic measurements of heat dissipation in photosynthetic complexes.

6) *The model based on radical pair mechanism (Fig. 5.14) seems to be oversimplified, therefore the results of the numerical simulation (Table 5.1) should be taken with caution. I see the weakness of the model based on which the NPQ and PPE (photoprotective effect) were calculated in the following points* ***The RC becomes closed if either the donor (via Ia+) or the acceptor (via PheoA) sites (or both) will be blocked*** *(for bacterial systems see Kis et al. Nature Sci. Rep. 12:14298, 2022). However, due to their different redox states, not all closed RCs are capable to form radical pairs.* ***The model in Figure 5.14 is insensitive to this fact****.*

   It is true that closing the reaction centres via oxidation of the primary donor Chl is not taken explicitly into consideration by the model. This is done intentionally because, unlike in bacterial systems, $Pheo^-$ and P^+ are short-lived in healthy fully-functional PSII due to fast and efficient charge recombination and electron donation from TyrZ. I agree that the model may not correctly represent the system behaviour under all conditions and must be used with caution.

7) *The conditions of dark adaptation and closed PSII RCs of Arabidobsis are hard to fulfil simultaneously.* ***Do you think that these conditions are achieved in the Fm state of the fluorescence induction kinetics? In which redox state is the RC closed?***

Yes, with respect to NPQ, it is possible to achieve closed RCs and maximal fluorescence in PSII before the onset of NPQ in Arabidopsis (but not necessarily in other species). This is the premise of the standard and widely PAM fluorometry technique for measuring NPQ. F_m is first measured by a short saturating light pulse (typically < 1 s). NPQ is then induced by continuous illumination (minutes) and calculated as NPQ = $F_m/F_m' - 1$.

8) ***What are the states and functions of the two radical pairs connected in series in the model (RP_1 and RP_2)?***

As Prof. Maróti correctly noted, the kinetic model is simplified and the kinetic compartments may not strictly correspond to separate physical entities such as different redox states of the RC. According to Szczepaniak et al. (2009, Biophys. J. 96:621), RP_1 can be assigned to P^+Pheo^- and RP_2 is a relaxed state of the same radical pair.

9) ***No traps of the excited bulk Chls (excitons) are considered (carotenoids, singlet-triplet transition, exciton-exciton annihilation, etc.) which can compete with the radical formation in the RC.***

We have omitted other processes that can trap antenna excitons because these are not supposed to play a significant role under physiological conditions. This is discussed in the relevant paper (Lambrev et al. 2012).

10) ***^{3}Chl should not be taken as a final product in the model*** *(the photodamage is attributed to ROS) but a pool of Chl triplets with inflow (from antenna and radical pair mechanism) and outflow spontaneous deactivation, triplet-triplet annihilation, quenching by carotenoids and/or by triplet oxygen) should be involved.*

While it is true that photodamage is ultimately caused by ROS, we assume that the main route of ROS formation in PSII is via ^{3}Chl formation in the RC (Vass 2011, 2012). Therefore, it is sufficient to calculate the yield of the precursor ^{3}Chl for the purpose of establishing the relationship between photodamage and PSII fluorescence (NPQ). Downstream processes of ^{3}Chl deactivation and ROS formation are certainly crucial for the final result (photodamage) but not relevant to NPQ (which only deactivates ^{1}Chl) and outside the scope of the model. On the other hand, these must be considered to quantify the photodamage in absolute terms, and not simply relative to NPQ. For example, when calculating the rate of photodamage in LHCII (Lingvay et al. 2020, Front. Plant Sci.), we have considered these processes (spontaneous inactivation, quenching by carotenoids and by triplet oxygen).

11) ***The antenna detachment inherently modifies the organization and the circumstances of energy migration.*** *Consequently, the model should be changed, as well (probably from "lake" to "domain" and/or to "separate" units, see below). The highly qualitative treatment (page 70-71) is obviously not satisfactory.*

I agree that antenna detachment may alter the dynamics of energy migration in ways that are not easily predicted. However, I should stress that the main purpose and conclusion of the modeling was to demonstrate that the presumed linear relationship between NPQ and photoprotection breaks down when antenna detachment takes place, precisely because the circumstances of energy migration are altered.

12) The concepts of reaction rates and reaction rate constants are not properly handled (they are commuted).

I am not aware of commuting reaction rates and reaction rate constants (I understand the difference) but if there is such an instance somewhere in the text, I apologize for the mistake.

3. "Far-red" chlorophylls in LHCII

13) According to thesis 2b, the "far red" chlorophylls of long fluorescence decay (large lifetime of excited state) are permanently present in PSII and are part of the photoprotective mechanism by deceleration of the migration and therefore of the funnelling of the excitation energy from the bulk to the RC via uphill transfer. They are considered as signature of NPQ. However, this mode of action is against the effective light harvesting function of LHCII and the concept of RC beeing a deep (effective) trap for the excitons. ***By what stoichiometry and spatial arrangement of the "red" chlorophylls in LHCII can be achieved the delicate balance between these contradictory functions in vivo? How can we understand the nature of low-energy excited states in efficient light collection of photosynthesis?***

I certainly agree that the existence of far-red chlorophylls in PSII is against the effective light-harvesting function. However, these states are not permanently present in PSII but are only formed in the quenched state where the light-harvesting function efficiency is reduced in favour of light energy dissipation. Thus, as Prof. Maróti correctly observes, the function of the far-red states is precisely to reduce the efficiency of light harvesting and the number of excitons reaching the RC.

14) In fig. 5.10 the far-red forms have much different spectra than Chls: very broad and complex. Do they correspond to single species but with complex energetic structure?

Absolutely, the far-red emission is broad, complex and unlike Chl excited states. However, these features are typical for mixed quantum states having contribution of both localized excitons and delocalized charge-transfer states. Whether the SAES correspond to single species, I believe that these states display large heterogeneity that in part causes the (inhomogeneous) broadening of the spectra.

15) It is confusing to plot the normalized spectra and not the relative amplitudes of the components.

I agree. The normalized plots lose information that could be useful to the reader.

16) The sequential model is essentially a branched model with elements coupled in series and parallel. ***Why the deactivation processes of Chl* different from those CHI*s which transfer the electron excitation energy to far-red-Chls were not considered?*** *They may compete with reactions to and within the CTs.*

We did include deactivation rate equal to the rate in unquenched LHCII. Prof. Maróti's comment is correct – there can be another deactivation channel competing with the transfer, essentially another hidden quenching mechanism. However, the key finding of our model was that it could explain the quenching by CT states without additional hidden mechanisms. This fact had evaded other researchers at the time.

17) *"...the sequential model...explicitly defines the CT states..." Rather the experiments than a model can prove the CT states.*

Yes, the existence of CT states is inferred from earlier experiments, not from the model.

4. State transitions

18) *Naively thinking, the balance of the excitation energy between the two photosystems will remain unchanged unless the spectral composition of the excitation persists. However, state transition does take place, when the intensity (low 4—). high) and not the spectrum of the illumination changes. Why?*

Generally, state transitions are activated by the redox state of the plastoquinone pool, which can be altered by different conditions and processes, not necessarily a change in the spectral composition of the excitation light.

19) *When PSII is overexcited then multiple LHCIIs can associate with PSI forming a well-characterized PSI—LHCI—LHCII supercomplex. Based on your model-experiment, can you identify the predicted multiple binding sites of the "additional" trimeric LHCII (thesis 7)? What was the role of LHCI in the excitation energy transfer from LHCII to PSI RC in your (model) experiments?*

Our experimental data lacks the resolution to establish whether LHCI mediates transfer from LHCII and to infer its binding position. A recent study citing our work has addressed this very question: "The role of LHCI in excitation-energy transfer from LHCII to Photosystem I in *Arabidopsis*" (Schiphorst et al. 2022, Plant Physiol. 188:2241). The authors show, using LHCI-deficient Arabidopsis, that LHCII can transfer energy to the core without LHCI but the transfer is twice as fast with LHCI present, suggesting that LHCI mediates excitation transfer from loosely bound LHCII.

20) *Table 6.8: How were you able to calculate the quantum yield of photochemistry based on the expression given in the legend?*

Unfortunately, the footnote to table 6.8 has a misplaced parenthesis making the formula incorrect. The correct expression ($1 - \tau/\tau_0$) is given in the main text.

21) *Kinetic scheme (Figure 6.30 A) and fluorescence lifetimes (Table 6.7): According to the model, the weakly bound LHCII (W) transfers excitation energy to the trap (PSI-LHCI core) with rate of (300 ps)$^{-1}$, although no corresponding component of the fluorescence decay can be seen. The major components appear with lifetimes of 20 ps and 80 ps.*

The fluorescence decay lifetimes are not equal to the inverse rate constants but are eigenvalues of the rate equation matrix. The transfer from LHCII(W) to PSI is the main process contributing to the 270-ps decay component. In the absence of this transfer, the fluorescence lifetime of LHCII should be in the nanosecond range.

22) *Thesis 7 says that "LHCII is an efficient antenna for both photosystems" because of state 1-state 2 transitions which regulate the balance of excitation energy distribution between both photosystems via lateral diffusion of a mobile fraction of LHCII. This is particularly true under low light conditions. However, state transitions tend to be suppressed under high-light conditions in plants (Oxborough et al.1987). The regulatory function of LHCII seems to contradict the photoprotective role which is one of the most important functions of the LHCII. Can you comment on that?*

Thesis 7 does not mention state transitions and was not intended to. We believe that LHCII can serve as antenna for Photosystem I in the stromal thylakoid regions even in state 1. This has been confirmed by more recent studies. Excitation balancing and photoprotection are two separate but not mutually exclusive functions of LHCII.

5. Energy transfer in LHCII and size of the PSU

23) *Thre are several models of antenna organization (for bacteria, see de Rivoyre et al. BBA 1797,1780-1794 (2010)): (a) "lake model", characterized by perfect connectivity where energy movesfreely between constituent units. This model is often used for PSII. (b) "puddle model" (or model of separate units), an extreme case in which excitation energy absorbed by antenna chromophores is always transferred to the same reaction centers. (c) "domain model", where two reaction centers are close to each other but the groups of two are not connected to each other. This model is well suited for scenarios in which dimeric aggregation of reaction centers exist.* ***Which model do you use****?*

The lake, puddle and domain models were developed before structural knowledge of the thylakoid membrane components and their supramolecular organization was available. Neither of these models completely represents light harvesting. According to the current understanding and the available structural and spectroscopic data, light harvesting in the granal thylakoid membranes has features of both the domain and lake model, in state 1 as well as in state 2.

24) *The essential feature of the light harvesting in the antenna is the energy funnel to the reaction center biased by the random walk of the excitation. They contribute to the irreversibility of the trapping of the energy at the reaction center. Here, however, the major photochemical step, the charge separation in the RC was omitted. In lack of the efficient trap by the RC, the excitons can visit much more sites during its long lifetime than in the presence of photochemistry. This will lead to* ***overestimation of the size of the PSU in lack of suited corrections****.*

The calculations of the functional domain size were primarily based on measurements with LHCII aggregates, which lack RCs and where no photochemistry takes place. These estimates can only give a rough idea of the PSU size in the thylakoid membranes. It is undisputable that the RC acts as an efficient trap for excitations and excitations can migrate further in the absence of a RC – I see no conflict in these statements and no basis for corrections.

25) *Our understanding of the structure and function of domains and PSUs has become highly sophisticated by recent results from experiments (e.g., high-resolution crystallography) and theory (e.g., statistical models) (see for bacteria Maroti et al. Nature Sci. Rep. 10:14012 (2020)).* ***What do you mean structural and functional domain sizes? How can the functional domain size be larger than the structural domain size (page 120)?***

I define the functional domain size in page 120 as the number of Chls that excitations can visit before decaying to the ground state. The functional domain size is thus related to the exciton diffusion radius. Obviously, the functional domain size cannot exceed the structural domain size, that is, the total number of chlorophylls present in the given structural domain. In practice, the functional domain size is smaller and in the case of PSII-LHCII supercomplexes, it is limited by the finite rate of energy transfer between pigment-different protein complexes. In 2011, I calculated that the functional domain size of LHCII aggregates and thylakoid membranes is equivalent to about 15-30 LHCII trimers. Several more recent studies are in agreement with these numbers.

26) ***I have concerns about the numerical evaluation of the data.*** *Dividing K_{SV} by τ_0, I will obtain k_q values different from the ones in Table 6.6. I have concerns about the numerical evaluation of the data. According to my calculations, the numerical values of the first, fourth and seventh rows (different environments) in Table 6.6 do not obey the simple expression $k_q = K_{sv} / \tau_o$ given in the dissertation. Assuming that the measured Stern-Volmer constants, K_{sv} and the fluorescence lifetimes, τ_o data are correct, then I will obtain k_q values different from those given in Table 6.6: The functional domain sizes given in the table could be wrong. The deviations are not negligible. Did I miss something in the "control-calculations"?*

This is true – as it is, table 6.6 is somewhat confusing. The average lifetimes are amplitude-weighted, as used everywhere else in the dissertation:

$$\tau_{av} = \frac{\sum_i a_i \tau_i}{\sum_i a_i}$$

However, k_q is calculated using intensity-weighted average lifetimes, hence the difference:

$$\bar{\tau} = \frac{\sum_i a_i^2 \tau_i^2}{\sum_i a_i \tau_i}$$

I believe it is appropriate to use intensity-weighted lifetimes in the context of the Stern-Volmer formalism. I apologize for not explaining the difference in the text.

27) *The functional domain sizes of LHCII in Table 6.6 were calculated based on the assumption of identical light harvesting antenna systems of the species with only difference of the interunit connectivity. Do you think that this condition was fulfilled?*

The functional domain sizes are expressed in units of LHCII because they are calculated based on LHCII trimers as a reference. Obviously, the entire thylakoid membrane consists of various other pigment protein complexes and the expression only serves as a simple guideline that allows the comparison of the domain sizes in thylakoids and in LHCII aggregates estimated by the same type of measurement.

28) ***What is the type of quenching: static (association) or dynamic (diffusion controlled)?*** *The Stern-Volmer analysis of the fluorescence quenching in different molecular environments clearly indicates the lack of diffusional limitation. You obtained k_q (the bimolecular quenching rate constant) values as high as $1.1.10^{14}$ $M^{-1}s^{-1}$ (Table 6.6). For diffusion-limited quenching (i.e., quenching in which the time for quencher PPQ to diffuse toward and collide with excited ChI in the LHCII is the limiting factor, and almost all such collisions are effective), the bimolecular quenching rate coefficient, derived from the Stokes—Einstein relation, is given by $kq = 8RT/(3\eta)$, where R is the ideal gas constant, T is temperature and ri is the viscosity of the solution. In water (η = 1 mPa-s, T = 300 K), $kq = 7.10^9$ $M^{-1}s^{-1}$. This is orders of magnitude less, than the observed bimolecular rate constant.* ***What mechanism (different from diffusion) can assure the observed very high rate of the quenching? Perhaps, a different and more complex view should be applied (see the next point)?***

This is a reasonable point. The observed quenching constants seem too high for a pure dynamic quenching process, however, not necessarily orders of magnitude. The bimolecular quenching rate constants, for example for quenching of Chl by benzoquinones, are $3 \cdot 10^{10}$ $M^{-1}s^{-1}$ and consistent with a diffusion-limited process (e.g. Gazdaru 2001, J. Optoelectr. Adv. Mat. 3:145). However, the effective quenching rate constant for a connected domain of 1000 Chls (~25 LHCII trimers) can be up to 3 orders of magnitude higher. The question of static

vs dynamic quenching is considered in Appendix A in the article on domain size (Lambrev et al. 2011), where we show that within certain limits, strong static quenching can be approximated to the Stern-Volmer formalism for dynamic quenching.

29) *The central idea of the determination of the functional domain size in the dissertation is the linear proportionality of the bimolecular rate constant of the fluorescence quenching to the domain size. Can you offer me evidence of that?*

We treat this question in Appendix B of the above-mentioned article by way of kinetic model simulations. The simulations demonstrate that in the case of sufficiently strong quenching, there is a quasilinear relationship between the observed quenching rate constant and the rate of energy transfer between LHCII, i.e. the domain size. However, considering all assumptions of the method, we conclude that "Our experimental approach at present does not guarantee reliable estimation of the domain size in all foreseeable scenarios—there are special cases where the underlying theoretical assumptions of the method are not satisfied."

30) *The drop of anisotropy of Chl fluorescence is a sensitive indicator of the transfer of electron excitation energy within the pigment bed. The method is particularly suited to estimate the orientation of the Chls in the aggregates and the diffusion length of the exciton.* ***Do you have any data (either from your lab or from the literature) supporting your results on excitation energy transfer in LHCII?***

Fluorescence anisotropy can reveal additional valuable information about energy transfer, especially isoenergetic transfer, e.g. between monomers in the trimers, which cannot be detected otherwise. We have performed fluorescence anisotropy measurements of LHCII trimers at room temperature using a synchroscan streak camera, where we found that the anisotropy decays with a lifetime of 3-4 ps and interpret this as representative of the intermonomer energy equilibration time. Du et al. (2005) studied energy transfer from Chl b to a by time-resolved fluorescence anisotropy finding transfer times 0.3 ps and 5-6 in good agreement with our results. A more detailed polarized transient absorption study was done by the group of van Grondelle at 77 K (Marin et al. 2012). We have considered their data in the modelling of energy transfer.

31) ***May I ask you to estimate (or to measure) the rate constants of processes (photochemical trapping and losses in forms of fluorescence, heat, etc.) competing with the energy transfer*** *in the antenna of any of your systems performing photochemistry (see the model-calculated values of a truncated system in Table 5.1)?*

Rather than answering this question in detail, I would like to refer to our recent publications, where we have made such estimations about LHCII (Lingvay et al. 2020, Front. Plant Sci.) and PSII (Akhtar et al. 2022, J. Chem. Phys.). Below are approximate values for the rate constants of various losses in LHCII:

Radiative rate constant	k_r	0.06 ns^{-1}
Nonradiative deactivation rate constant	k_d	0.1 ns^{-1}
Rate constant of intersystem crossing	k_{isc}	0.1 ns^{-1}
^{3}Chl→Car transfer	$k_{T\text{-}T}$	2–10 ns^{-1}
^{3}Chl→O_2 transfer	k_{ox}	2×10^9 $M^{-1}\,s^{-1}$

6. Exciton dynamics

32) *All the absorption changes spectra used to construct the 2D plots are normalized and amazingly smooth probably due to the very high light intensity of the measuring beam.* ***How large are the intensities of the pumping and probing pulses?***

In our earlier experiments we used pulse energies in the order of 15 nJ. However, over time the experimental setup was continuously optimized with the goal to reduce the intensity and the more recent data in the dissertation were obtained with pump pulse energies of 0.5 nJ and much lower probe intensities. I am not aware of any other group having published results with such low energies. That said, the data are noisy, which may not be obvious from the processed 2D contour plots.

33) *I'm wondering whether the bright detection beam can cause-any disturbing effects like singlet-singlet annihilation or other ways of (e.g., triplet) quenching of the excitons?* ***How low the laser pulse energy should be kept to avoid the exciton-exciton annihilation? Can you offer me the actual absorption change not in relative units but in absolute units (mOD)?***

This is a highly relevant question and the very reason why we have made every effort to minimize the beam intensity. Earlier experiments clearly suffered from annihilation effects; however, these were eliminated using low-energy pulses and we were the first to publish annihilation-free 2DES data of photosynthetic proteins. The absolute transient absorption signal obtained with 1 nJ pulses is about 1 mOD at the maximum ($\Delta A/A = 0.3\%$). Under these conditions, annihilation is negligible. The probe beam, on the other hand, is only a fraction of the energy of the pump and spread over a very broad spectral range where absorption is low.

34) *In your recent paper (Do et al., EPJ Web of Conferences 205, 09038, 2019), you estimated that the excitation laser spectrum covered nine excitonic states in LHCII. In the dissertation, you considered eight instead of nine excitonic states in your model.* ***What could be the reason to omit the state centred at 659 nm between the states of 655 nm and 664 nm****?*

The pulse generation technique used in these experiments gave only a limited bandwidth and the signal-to-noise ratio was not sufficient to resolve all states in the Chl *b* region. Fitting models with more than eight excitons gave an infinite number of solutions.

35) *You argue that "Model A has more states decaying in 0.1-1 ps and provides better fit" than model B. However, the data in Table 6.3 do not coincide with EET rates for 7; = 200 fs given in Fig 2 of Do et al. (2019).* ***Can it be said that the model used in your previous publication was not appropriate enough?***

I would like to remark that the mentioned conference paper, to which I am not a corresponding author, is neither included, nor cited in the dissertation. One could argue that, by definition, any model is “wrong”, as long as it does not capture all aspects of the real system it mimics. The models included in the dissertation are the same as in the peer-reviewed journal article by Do et al., published in the same year. These models are more complex, as they also fit cross-peaks at time zero.

36) ***I see contradicting conclusions in the two works when the results are compared with the predicted slow exchange rates of Renger et al. (2011).*** *In the dissertation, you argue that "the EET times are in line" with those of Renger's calculations, although the slowest energy transfer was 13 ps. In paper of Do et al. (2019), however, similar (9 and 25 ps) or much slower (>100 ps) transfer*

times were given but the conclusion (despite the slower transfers) was opposite: "the rates that we obtain are faster than structure based theoretical model by Renger et al." The 100 ps transfer time was not slow enough to match Renger's expectation?

Although they seem contradictory, both conclusions are partly correct and not mutually exclusive – we find individual rates to be faster than in Renger's calculations, yet the overall timescales of energy transfer are comparable. I should reiterate that you refer to a conference paper with a preliminary analysis. A more detailed analysis and explanation was given in the subsequent full-length article.

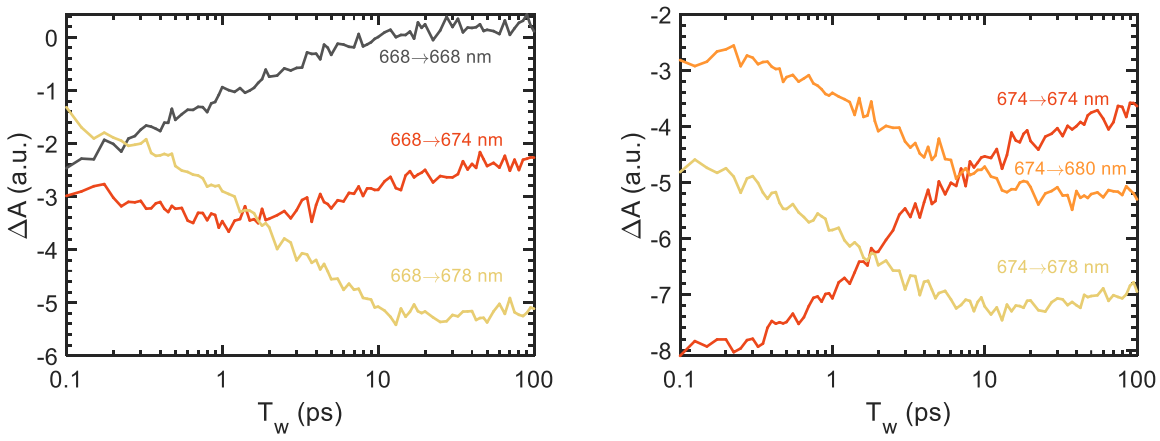
37) *The local pigment excitation energies are tuned by the electrostatic interactions with the protein and solvent environment. The interaction between the energetically varying local states results in a ladder of excitonic states, where the higher energy states are localized toward the peripheral antenna complexes, while lower energy excitons are close to the photosynthetic reaction center. The two-dimensional electronic spectroscopy (2DES) is a suited tool to determine the spatial and temporal structure of the exciton states in the pigment bed (see also the FMO protein of green sulfur bacteria).* ***I'm wondering whether the candidate was able to map out the excitonic level-to-level transfer in LHCII? What are the strongly coupled 8 states used in the dissertation?***

It is indeed possible to assign spectral forms resolved in the 2DES experiments to individual Chls, if their site energies are known. Taken together the literature knowledge based on mutational studies and structure-based calculations, we have made assignments of the Chl a exciton states in our energy transfer model. A schematic drawing of the level-to-level transfer with Chl assignments is given in Figure 6.15.

38) ***I don't see any sub-picosecond kinetics from which the fascinating 2DES maps could be constructed. Why isn't a single kinetics presented in the dissertation?*** *This would be interesting to me to know whether an oscillation at the very beginning of the trace (up to about 100 fs) is observable, which was discovered by Vos back in the early nineties (and were not referred to in the dissertation (page 82)): Vos et al. Direct observation of vibrational coherence in bacterial reaction centers using femtosecond absorption spectroscopy, PNAS 88,8885-8889 (1991) and Vos et al. Visualization of coherent nuclear motion in a membrane-protein by femtosecond spectroscopy, Nature 363,320-325 (1993). These and related phenomena initiated heavy discussion among theoreticians. Is there a longer-lived quantum coherence between excitons in photosynthesis or the energy transfer in the pigment bed is controlled exclusively by promptly excited vibrations?* ***What is your opinion about the possible role of interexciton coherence in excitation energy transfer in photosynthesis?***

Firstly, I should apologize for not including raw kinetic traces in the dissertation – this would have indeed been useful. However, oscillations along waiting time would not be resolved in the included experimental data mainly because of the sparse and logarithmic sampling of delay times. We have observed weak oscillations in other experiments but have consciously avoided those because: 1) the experimental setup is not well suited to study excitonic coherence, as we don't separate rephasing and non-rephasing spectra; 2) coherent oscillations in LHCII have been published by other groups already. We have recently reported oscillations in Photosystem I consistent with exciton-vibrational coherence (Akhtar et al. 2021, JACS) – the largest complex where such oscillations have been detected. The question of the role of exciton coherence in energy transfer has been highly debated by specialists. The currently emerging consensus is that the long-lived coherences originally observed in FMO and previously assigned to interexciton coherence, most likely originate

from vibrational modes in the electronic ground state (Cao et al., Sci. Adv., 2020). Nevertheless, quantum effects are found in various aspects of energy and electron transfer.



Waiting-time dependence at selected points of the 2D electronic spectra of LHCII at 77 K.

39) *A general question to the end: the focus of the treatment of exciton dynamics in the antenna was set on the trade-off between increased absorption cross-section and quantum efficiency of PSII (see the adaptation of green alga Bryopsis corticulans).* ***Is this question so essential to the alga indeed or we like to increase its importance?*** *The overall yield of the photosynthesis, namely, is very low (a couple of % only) and the bottleneck is certainly not the high yield of photo-utilization in LHCII.*

There are two aspects to this question: 1) Is the high yield of photo-utilization of LHCII essential to the alga? Yes, without doubt, optimizing the absorption spectrum to match the light available in the habitat is the evolutionary driving force for the spectral adaptation of LHCII. 2) Is the trade-off, i.e. degrading of energy transfer rate in the algal LHCII important? No, in my opinion the minor loss of photo-utilization efficiency is not a bottleneck and is inconsequential to the algal physiology and fitness. However, understanding these trade-offs might be valuable in future attempts to engineer light-harvesting systems for specific purposes.

Minor comments

40) *I would not use the phrase "on the role of..." in the title as it expresses the feeling that your mind drifted back and forth, and the dissertation might be less concentrated, unfocused, and undirected. To avoid that, a more definite statement in the title would have been chosen.*

I would accept the suggestion.

41) *The formal classification of each thesis statements is divided into paragraphs. However, statements 5 and 7 include a single point only with indication to additional statements.*

I agree with the comment.

42) *About the shot history of the Forster theory: the referred monograph from 1965 was a late consequence of a much earlier published paper in Naturwissenschaften in 1946 outlining the quantum-mechanical behaviour of the transfer of electronic excitation energy between two molecules*

in a solution. This breakthrough work in spectroscopy explained the transfer of electron excitation energy between two molecules non-radiatively.

I agree with this statement.

43) *According to the original classification of the strength of coupling (very weak, weak, and strong, Forster 1965), the very weak (dipol-dipol) interaction corresponds to the weak coupling you mentioned in your dissertation. If I'm not wrong, I see some deviations of the definitions between you and Th. Forster.*

I agree with the comment, Förster subdivides the "weak coupling" regime into "weak" and "very weak" and explains that energy transfer between strictly localized states only applies in the latter case.

44) *$f_D(\nu)$ denotes not the fluorescence quantum yield of the donor in the absence of acceptor, but the normalized fluorescence spectrum of the donor.*

Indeed, the symbol is mistyped, it should be ϕ_D instead.

45) *$\varepsilon_A(\nu)$ denotes the absorption spectrum of the acceptor and not the fluorescence of A as could be deduced from the not clearly worded sentence.*

Yes, $\varepsilon_A(\nu)$ denotes the absorption spectrum of the acceptor. Apologies, if the sentence wasn't clearly worded.

46) *What is C in the expression of the Forster radius (Eq. 2.1.10)?*

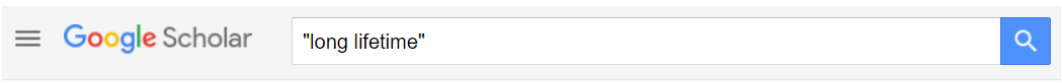
C is a proportionality constant. It can be expressed as $\frac{9000(ln10)\phi_D}{128\pi^5 N_A}$ (Scholes 2003, Annu. Rev. Phys. Chem. 54:57-87). As the Background chapter was meant to introduce the basic concepts used in the dissertation, rather than serve as an exhaustive reference for well-established theories, many details have been omitted. I admit that this is one occasion where a more complete expression could be given.

47) *The sentence on page 28 is obviously not correct: "On the acceptor side of PSII, electrons are transferred from Pheo to QA in 200-300 ps and then to the two quinones Q_A and Q_B…"*

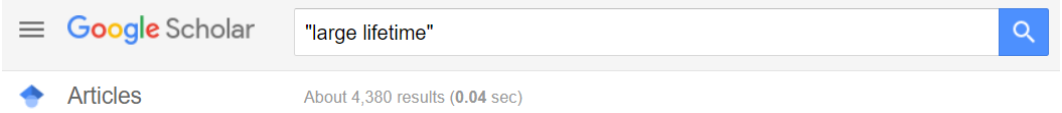
I apologize for the mistake. After Q_A electrons are transferred to the secondary quinone Q_B.

48) *I do not dare to judge the English of the dissertation except of two (very frequently used) phrases: According to my view: the fluorescence decay can be either short or long, but the fluorescence lifetime can be either small or large (and not oppositely, the fluorescence lifetime cannot be short or long). This why the quantity "lifetime" was introduced to measure the decay.*

49)



50)



I must submit that the phrase "*short/long lifetime*" is perfectly valid from a linguistic and scientific perspective. As the fluorescence lifetime is expressed in units of time, the qualities

short/long are more precise than *small/large*. That is why *short/long* are the preferred descriptions in the literature (as demonstrated by a simple online search).

51) *I would say "the lifetime of the excited state" instead of "excitation lifetime" which could be confused easily and erroneously with "the duration of the excitation".*

Thank you for the suggestion. I would remark that the terms "*duration*" and "*lifetime*" are rarely, if at all, used interchangeably in the specialized scientific literature.

52) *Compared to the promising results on 2DES, theses 1 and 2 include modest statements. They are based on mainly routine investigations which were carried out with care and require skill and expertise. The outcome of most of the experiments is not very much surprising and fit to the general expectations. E.g., the statement about the dependence of the structure, self-regulation, and the excitation properties of the LHCII on the environment is not exceptionally new and unexpected (the opposite statement would be surprising), although it should be worked out properly with tiresome efforts.*

I would argue that an essential part of the scientific method is testing hypotheses or expectations by careful experimental investigations. The results summarized in theses 1 and 2 were met with surprise and at times disbelief by some members of the community when they were first announced. Today, they could be seen as trivial.

53) *Most of my questions with stronger criticism are related to theses 5-7. I have the greatest concerns about thesis 6. According to my opinion, they are the weakest points of the dissertation. I hope my worries and uncertainty will disappear after the answer of the candidate and after the open discussion.*

Regarding thesis 5 ("LHCII is a spectrally tunable antenna"), I hope that my answers will disperse Prof. Maróti's concern.

Regarding thesis 6 (about functional domain size), I admit that the number stated "about 25 LHCII trimers" comes with caveats and in hindsight I agree that including this number in the theses may not be entirely warranted.

Regarding thesis 7 ("LHCII is an efficient antenna for both photosystems") – I would reiterate that a considerable body of literature has recently emerged that supports our findings.

Szeged, 28 February 2023

(Petar Lambrev)