Review of the PhD thesis by Gürkan Keşan entitled Excited States of Carotenoids and Their Roles in Light Harvesting Systems

The thesis combines an experimental and theoretical approach towards studying excited states of carotenoids. None of the approaches is easy due to the peculiar properties of the carotenoid molecules, and they become even more complicated for carotenoids containing a carbonyl group in their conjugated system. This adds another unusual excited state to several other states, the nature of which is still not well understood. Gürkan Keşan has managed to successfully overcome the difficulties connected with the study of both carbonyl and non-carbonyl carotenoids, as becomes obvious after reading this thesis.

The thesis consists of 7 papers dealing with carotenoids both in solutions and photosynthetic complexes, which are completed with an introduction and description of experimental methods. All the papers were published in respected journals and the applicant is first or second author in five of them and fourth author in paper 1 and 2. I was wondering why these two latter papers were included in the thesis, considering that the remaining five ones would be more than sufficient for an outstanding thesis, and the contribution of Gürkan Keşan to them is obviously more substantial.

The main experimental approach used in this thesis was femtosecond spectroscopy. It was used for the spectral characterization of excited states, and for the determination of excited state lifetimes or transfer times. In papers 3, 4 and 5, complementary information on e.g. ground and excited state geometries or transition energies was obtained by DFT and MD calculations. The thesis is written with care in good English and mistyping like "simulated emission" on page. 31 are rather rare. My only complaint concerns the quality of some of the figures in the Introduction and Experimental methods, which are reproduced in rather low quality and, more importantly, with labels typed using very small fonts that make reading them rather difficult. I can mention figures 1.3.1, 1.4.1 and especially 1.4.3 as three examples. However, the quality of the figures in the Research section is satisfactory.

Reading the thesis prompted several questions, including the following ones, which I would like to ask the candidate during the defense:

1) I did not find any errors or standard deviations for the reported lifetimes. Does it mean that the same values were obtained reproducibly?

2) As the candidate is experienced in quantum chemical calculations, he might be able to explain to me why cis-carotenoids exhibit the same excited state structure (with few exceptions including the cis peak) even if they do not exhibit any C_{2h} symmetry of all trans carotenoids and polyenes? Why, for instance, the S_1 state is forbidden for cis-carotenoids?

3) In paper 1 you proposed that the S_1 and ICT states of 8'-apo-beta-carotenal is one and the same state whose charge transfer character depends on the polarity of the solvent. Is it possible to generalize this conclusion to other carbonyl carotenoids? My impression was that in a newer paper 6 this claim was not stated so unambiguously for isofucoxanthin-like carotenoid.

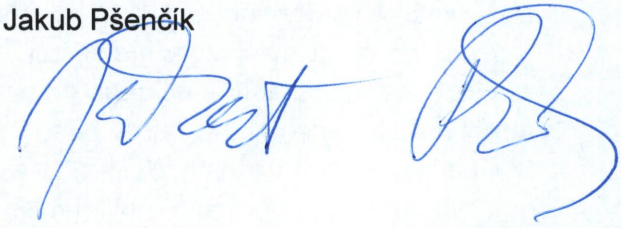
4) Do you know the mechanism of how different amount of water in the solvent causes formation of different types of aggregates (H-aggregates vs. J-aggregates), and why a different way of preparing the same mixture enhances a contribution of two spectrally different H-aggregates, as described in paper 2?

5) In paper 6 it was concluded that the charge-transfer character of isofucoxanthin-like carotenoid's S_1/ICT state is suppressed in order to achieve high light-harvesting efficiency. This conclusion is based on a fact that the contribution from the ICT state is much less pronounced in a slow EADS component connected with the decay of the S_1/ICT state in the CLH complex compared to the decay in acetonitrile. However, from Figs. 3.6.2 and 3.6.3 it seems to me that in the fast EADS component, which is connected with a more efficient transfer energy to chlorophylls, the ICT band contribution is rather strong even after subtraction of the contribution from Chl *a*. Can you comment on this?

In summary I can conclude that the submitted thesis clearly proves the ability of Gürkan Keşan to perform a high quality research. His dedication to science and a broad field of interests are apparent not only from the content of the thesis, but also from the fact that he published another 11 papers between 2012 and 2016, which dealt with other topics than carotenoids. Consequently I gladly recommend to award Gürkan Keşan with the title PhD.

January 30, 2017

Jakub Pšenčík



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Report on PhD thesis

Please find below my report on the doctoral theses "Excited States of Carotenoids and Their Roles in Light Harvesting Systems" by Gürkan Kesan.

The thesis presents work conducted by Mr. Kesan under the supervision of prof. Tomáš Polívka and his co-superviser prof. Cemal Parlak in form of seven papers published in impacted international journals. Two of these papers have Mr. Kesan as first author, and he contributed to both experimental and theoretical parts of the work in essentially all seven papers. The work conducted for this thesis tries to shed light on the properties of excited states of carotenoids in the context of light-harvesting and energy transfer as a primary processes of photosynthesis.

The thesis starts with a very informative overview of the process of photosynthesis and of the two major groups of pigments involved in light-harvesting and energy transfer, namely, the chlorophylls and carotenoids. Mr. Kesan usefully compiles the properties of a list of carotenoids from both literature and the papers on which the thesis is based into an informative table. Energy transfer mechanisms in photosynthesis involving carotenoids a briefly mentioned. The section "computational methods" discusses the difficulties in treating properly the excited states of carotenoid molecules in the framework of quantum chemistry. Calculations of the frequency of C=C stretches of various carotenoids are presented for estimation of the effective conjugation length of non-linear carotenoids. Introductory section of the thesis ends with a short overview of experimental methods, as the author was actively involved both in the experimental and theoretical works.

Overall, the introductory section is written in good scientific English with only few grammatical slips. Writing skills are certainly demonstrated to a satisfactory level. The thesis has a value not only as a report on specialized research, but also as a reference to number of carotenoids and their properties.

The research part of the thesis consists of seven papers presenting work on carotenoid excited state properties and carotenoid-chlorophyll energy transfer published in international peer reviewed journals of good quality. Research section is summarized in the Conclusion session of the thesis, were the most important results of the seven papers are briefly discussed. As a

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collection of published papers, the research part has already withstood critical scrutiny of specialists. With recent changes of the general publication landscape and habits, the quality of peer review process cannot be straightforwardly assumed based on, say, impact factor. In this respect it is important to mention that all papers presented in this thesis are published in journals in which carotenoid and photosynthesis research is usually published, and this ensures that the referees were indeed specialists, most likely surpassing in qualification the author of this report.

Mr. Kesan has a good publication record of other eleven papers and the papers presented in this thesis are chosen to present a coherent body of work. This justifies the style of the thesis (introduction + papers). Mr. Kesan's contribution to the papers seems to be essential in several respects; most notable is his involvement in both experiment and theory. Theoretical contributions consist of state of the art quantum chemical calculations and their analysis.

The work does not raise any obvious questions beyond those, which stem from the evaluator's special interest in the topic. I believe that the presented thesis meets international standards of PhD theses, and both the published body of work, and the thesis demonstrate candidate's research skills to an extent required for PhD.

Tomáš Mančal

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To Prof. Dr. Tomáš Polívka
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Subject: Report on PhD thesis entitled „Excited States of Carotenoids and Their Roles in Light Harvesting Systems" by Gürkan Keşan

Vienna, 26th of January 2017

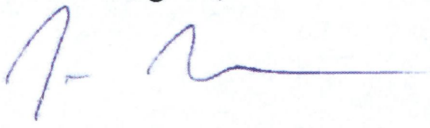
Dear Professor Polívka,
It is with pleasure that I send you this report on the PhD thesis of your student Gürkan Keşan. The thesis is built around the impressive number of seven papers, all published in international and esteemed journals. The common theme of all publications and therefore the thesis is ultrafast dynamics in carotenoids. As summarized elegantly and in an easily accessible way in the introduction, carotenoids are one of the two essential building blocks of natural light harvesting complexes. While the polyenic structure of carotenoids seems simple, they fulfill numerous roles and functions, all concisely reviewed in the introduction to the thesis. This multi-functionality of carotenoids derives from an ultrafast and intricate energy deactivation system, the details of which have been at the center of an ongoing scientific debate, spanning back to the advent of ultrafast spectroscopy in the early nineties. Faced with convoluted and ultrashort-lived transient species as typical for carotenoids, one approach is to employ specific and evolved spectroscopic techniques such as pump-dump-probe, four-wave-mixing on excited states, or two-dimensional electronic spectroscopy. This trend to ever more complicated techniques was followed extensively until the early 2000s and is still pursued today. Alternatively, and impressively applied in the present thesis, one can apply standard spectroscopic methods but investigate carotenoids with exceptional (paper 5) and varying structural properties (paper 3) or even unknown structure in different solvents (paper 4), employing time-resolved spectroscopy as a tool to suggest molecular properties. Carotenoid dynamics in a protein environment is another large field of research, this thesis makes interesting and valuable contributions to (papers 6 and 7).
Below I list a few suggestions for improvements:

1) Paper 3 especially rests on results from density functional theory, which is introduced in section 1.6. Statements such as "MRCI in combination with DFT gives more accurate results for excited state configurations [...]" should be qualified with respect to the applied methods such as CAM-B3LYP. Why is it okay to use these more approximate methods in the discussed cases, while more precise methods are available?

2) Global analysis is used for example in paper 2, but not discussed as a method in the introduction part of the thesis. As a widely used analysis tool, it would have been fair to review global analysis in its strengths and weaknesses in the introduction.
3) On a more general note: the thesis would benefit from and extension of Chapter 4. "Conclusions and Summary" to "Conclusions, Summary and Outlook". What other experiments could and should be done, building up on the presented findings?
4) Similar to point 3): what ultrafast techniques other than transient absorption spectroscopy would be beneficial for the systems studied in this work? Would better time resolution sub 100 fs be of interest?

In summary, I conclude that this thesis fulfills all necessary requirements for awarding a PhD and should be accepted for defense.

With best regards,



Jürgen Hauer