

Referee's report on PhD thesis:

Robert G. West

**Carotenoid Excited State Processes by Femtosecond Time-Resolved Pump-Probe
and Multi-Pulse Spectroscopies**

The presented thesis describes the work towards understanding complicated excited-state dynamics of carotenoids, mainly by the means of ultrafast, time-resolved spectroscopy. The work consists of two main parts, Introduction and Research Chapter, each splitting into for subsequent subchapters. The Introduction chapter is gradually presenting carotenoids in general, covering their relevance in Nature, which is followed by explaining the complicated manifold of carotenoid excited states. Further, the time-resolved (pump-probe and multi-pulse) spectroscopy is introduced, together with the methods of acquired data analysis with necessary fundamental theory.

This chapter is very well written with careful selection of phrases, making it clear yet precise and scientifically accurate. All the claims are well explained and carefully referenced with relevant and up-to-date references, placed at the end of the chapter. Together with the flawless language, it is very pleasant to read.

The second and main chapter, called "Research Chapter" is based upon four scientific papers, which are either already published (Paper II, III and IV), or submitted for publication (Paper I) in high-quality, peer-reviewed scientific journals. The author of this thesis is either first author (Paper I and II) or co-author of presented scientific papers, exploring in the depth the excited-state properties of carotenoids, mainly aiming towards carbonyl carotenoids (containing conjugated carbonyl group) with strong focus on understanding the nature of ICT state and its relation to the first excited state, the S1.

This chapter is then followed by a short (4 page) Summary and Conclusions, summing up the most important results of the presented papers.

Comments and questions:

I personally consider the Paper I (Chapter 2.1) the center point of this thesis and research done by the author. It showcases the potential of multi-pulse spectroscopy (and transient-absorption in general), and the potential of advanced data handling and simulations in revealing the complicated excited state dynamics of carotenoids, namely the illusive ICT state. And I'm impressed by the "cutting-edge" results as well as by the tedious work invested in this study, which represent the current frontier in the ICT research. In this chapter, the inner nature of the S1/ICT state is described as, or reaffirmed to be (Chapter 1.1) two parts of one potential energy surface, separated by a barrier. This is a bit confusing with the following text (Chapter 1.1, page 17, top) where the work by Vengris and coworkers is introduced, clearly identifying S1 and ICT state as separated states (Ref. 72). Furthermore, in the Summary and Conclusions it is also written (page 191, top), that the S1 and ICT states have been "clearly acting as separated states", seemingly contradicting the first (and as I believe the most important) research result of the author.

- Could the author please explain/clarify his view on the above-mentioned controversy about the nature of the S1 and ICT or S1/ICT states?

While focusing on the steady-state absorption and emission spectra, I was surprised by the fact, that the low-temperature emission spectrum is actually broader than the RT spectrum. This is rather unusual! The author's comment in the text reads "*Upon lowering temperature to 190 K (Fig. 2.1b), the absorption spectrum of fucoxanthin in methanol broadens, indicating that polarity-related features are enhanced at low temperature*" – what does this mean?

- Do you expect activating emission of other molecular conformers by lowering the temperature? How? Or is there another effect to explain the broader spectra at low temperature?

I was also thrilled by the results of the Paper III (Chapter 2.3), where the NON-conjugated acyloxy group on fucoxanthin derivatives (bFx and hFx) effectively deactivate the ICT state (and any polarity-induced effects). How is this possible? To my knowledge, it has been "always" believed, that only chemical groups conjugated to the main carotenoid's backbone are effecting its excited-state properties. The argumentation in Paper III refers towards similarity with previous work by H. Frank and coworkers with crocetindial (two symmetrical carbonyl groups) and diapocarotene (one carbonyl and one hydroxyl group, the actual Ref. 16), which analogy, I believe, is not correct. While the ICT signatures in crocetindial are indeed

missing, the effect is attributed to the symmetrical positions of both conjugated carbonyl groups, a phenomenon known for symmetrical carbonyl carotenoids (the effects of both carbonyl groups effectively cancel each other). However, here in the case of bFx and hFx, the auxiliary acyloxy group is not attached to the conjugated system of the carotenoid.

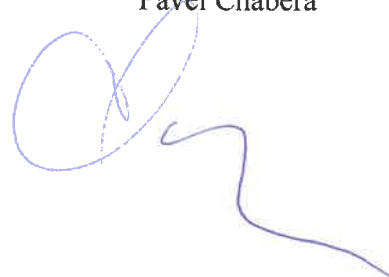
- How can electron withdrawing ability of non-conjugated acyloxy group compete with (and cancel out) the contribution of the conjugated carbonyl group at the other end of the molecule?

Here I would like to mention a mistake in the references numbering. It looks like Ref. 16 is not numbered and thus consequently, all the following references have a wrong number. This made me puzzled for a while as searching through the references was really tricky. Fortunately, the published version of the paper has the numbering flawless, thus one can use it as a guide. I would strongly recommend mentioning this in eventual errata.

In summary, I consider the thesis to be very well written and the results interesting and highly relevant. Therefore I recommend the thesis for defense.

Lund, June 7, 2018

Pavel Chabera





**Evaluation of Ph.D. thesis titled
“Carotenoid Excited States Processes by Femtosecond Time-Resolved Pump-Probe and
Multi-Pulse Spectroscopies”
by Robert G. West**

The thesis aims study of carotenoid energy states by measurement and evaluation of ultrafast time-resolved absorption spectroscopy; the energy states of fucoxanthin, its two derivatives and alloxanthin in solutions and in a diatom are explored. This effort has led to 3 already published papers in impact-factor journals where the PhD candidate is the first author in two of the papers, and to 1 submitted manuscript where the PhD candidate is the first author again. As a whole, the thesis represents a coherent work on the topic.

As for the formal aspects of the thesis, even if in the invitation-to-review-letter I am recommended not to comment this, I miss in the thesis clearly written aims and objectives, which should imply from the introduction and should be placed behind the introduction.

As for the factual aspects of the thesis, I will comment only the introduction and put some questions, since the published papers have already been peer-reviewed.

In the thesis, I have found some incorrect or strange (from my point of view) statements, which have led to following trivial questions:

- Page 2 – Has *Prochloron* chloroplast(s)?
- Page 7 – What “stable energy products for cellular processes” are manufactured in the reaction centers?
- Page 15 – When the S_1 and ICT (intramolecular charge transfer) states are considered as yet the same excited state species and when as already entirely different excited state species? What is qualitative/quantitative measure for the sameness/difference of/between the states?
- Page 28 – The first excited state of a carotenoid is not allowed by an excitation but what is meant by statement that the excited state is “considered a dark state”?

Then I have some “more scientific” questions, which have arisen from the fact that I am not expert in the field and I have not found the answers in the thesis. The questions are:

- Page 35 – While physical origin of the intramolecular charge transfer (ICT) state directly implies from its name, I would like to know what is the physical origin of the ground state intermediate (GSI).
- Page 37 – What were “average” intensities of the pump and probe pulses (in $\mu\text{mol (photons) m}^{-2} \text{s}^{-1}$) in the sample (some information is provided on page 114, 148 and 175) and what was duration of a typical experiment? If the intensities are high and/or

duration long, cannot the sample be destroyed by the light (once, usage of a sample stirrer is even mentioned)?

- Page 39 – In fact, any sample is inhomogeneous and then the linearity of A with respect to ϵ , L and C (Eq. 1.13) need not be valid (see Nauš et al. 2018, Photosynth. Res. 134, 345-355). Has a possibility of non-linearity of A been ever mentioned/discussed in the literature on the fast time-resolved absorption spectroscopy?
- Page 39 – If energy transfer from one molecule to another molecule occurs, assumption of the first order kinetics is not correct since the transfer depends on concentration of both the “species”. Would consideration of the second order kinetics significantly change the results?
- Page 42 – How have you quantified quality of the fit of a model to experimental data (the criterion) and which mathematical procedure have you used to determine the best estimates of the fitted model parameters?
- Page 42 – “... when multiple models produce the same fitting results” (i.e. about the same fit quality was obtained for models with different number of species and transitions between them), why you have not used any information criterion (e.g. the minimum Akaike information criterion estimate, MAICE) to decide, which of the models “better” describes reality (i.e., a smaller relative amount of information is lost when the reality is approximated by the model than by another model)?
- Page 43 – What is a general approach to formulate structure of the model to be fitted to experimental data? I mean, based on which facts you consider particular “species” and transitions between them in the model?

Regardless my previous questions, I recommend the Ph.D. thesis by Robert G. West for the defense.

June 8, 2018, Olomouc



doc. RNDr. Dušan Lazár, Ph.D.

Review of the PhD thesis by Robert G. West entitled Carotenoid Excited State Processes by Femtosecond Time-Resolved Pump-Probe and Multi-Pulse Spectroscopies

In this thesis, femtosecond spectroscopy has been used to study excited state properties of carotenoids. The emphasis was put on properties of the intramolecular charge transfer state of carbonyl carotenoids. The thesis is distinctive by application of a multi-pulse technique (three pulses instead of a standard pump-probe two-pulse scheme), which is experimentally demanding, and also more difficult to interpret. The benefit of such an approach is that it provides information which is otherwise difficult to obtain.

The thesis consists of an Introduction and Research Chapter based on 3 published and 1 submitted papers in renowned journals, with Robert West as first author in 3 of the papers. The Introduction was probably written under time pressure. Many sentences are difficult to understand (see, for instance, the second sentence on page 31, and my question no. 3), while others sound incorrect. If I limit myself to the page 31, I have to object to the statements describing the Dexter mechanism of excitation energy transfer. Firstly, it does not occur via a spin exchange, but by electron exchange. Secondly, the spin of the exchanged electron remains conserved. And thirdly, the Dexter mechanism does not account for a singlet or triplet electron transfer, as written in the same paragraph, but for a singlet or triplet excitation energy transfer. The number of inaccuracies and incorrectness in the Introduction significantly exceeds a level typical for doctoral thesis, and it includes mistakes even in fundamental relations of photophysics or basic facts about the photosynthesis. Absorbance does not depend on a volume, as claimed in the equation 1.13, and reaction centres do not provide stable energy products, as written on page 7. In fact, all the processes in reaction centres are reversible. As a non-native English speaker, I was reluctant to evaluate the language level of the thesis, however, I have to admit that it contains grammatical errors, including the use of a double negative, mistakes in subject-verb agreement, and frequent typing errors. Thesis introductions often serve as a first reading for new students, but I would not recommend it in this case.

The research part of the thesis, however, more than compensates for the unsatisfactory impression of the Introduction. Three-pulse time-resolved spectroscopy and advanced target analysis requires an experienced scientist with a deep understanding of photophysics in order to provide realistic conclusions. Robert West proved in his papers that he is such a scientist. His results led to a revelation of new information about the intramolecular charge transfer state in carbonyl carotenoids, its relation to the S_1 state and a role in light harvesting, and significantly improved the general understanding in this field. Other results include the effect of the triple bond in the backbone of the carotenoid molecule on its conjugated system.

At the defence I would like to ask several questions related to the text of the thesis:

1. On page 7 it is written that "Carotenoids exhibit efficient energy transfer upon excitation due to the fact that their energy transfer rate is faster than their deactivation rate." Is this an intrinsic property of carotenoids?

2. In Figure 1.3, phosphorescence of a carotenoid molecule is depicted among other relaxation processes. Are you aware of any reliable report of carotenoid phosphorescence? Is it more commonly observed than phosphorescence of chlorophylls, which is absent in the scheme?

3. Can you explain the meaning of the second sentence on page 31?

"When in closer proximity, intermolecular energy transfers have low dipole strength (Eq. 1.9) and thus low probability (Eq. 1.7) of occurring only if spin is not accounted for."

4. The information provided by Evolution-Associated Difference Spectra and Decay-Associated Difference Spectra is different, but the determined lifetimes should be the same, at least in an ideal case. In Figure 1.14, however, both methods provide different lifetimes for synthetic data. Can you explain this discrepancy? I think that the synthetic data can be considered to be an ideal case.

5. On page 37 it is written that "The mutual polarizations of the pump and probe were set to the magic angle of 54.7° to mitigate the effect of solvation dynamics." Does it mean that solvation dynamics can't be studied under the magic angle?

6. A single potential surface implicates either one state or two (or more) strongly coupled states. If I understand correctly conclusions of your latest paper, the S_1 and intramolecular charge transfer state are two different states sharing a single potential surface, and therefore must be strongly coupled. What is the nature of the coupling between the S_1 and intramolecular charge transfer state?

In summary, I believe that Robert proved in the Research Chapter of his thesis that he is capable of conducting an independent research. Therefore I recommend to award Robert G. West by the title PhD.

June 8, 2018

Jakub Pšenčík

