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Thesis report of Ph. D. Dissertation

by Valentyna Kuznetsova

named "**Time-resolved spectroscopy of light-induced processes**"

Overview. The thesis with the quite general title describes the photo-physical behaviour of a few carotenes and carotenoids. Certainly, the topic is important one and presumably heavily studied worldwide. Any new findings there may bring important breakthrough in various branches of science and therefore the analysis of photo-physical and photo-chemical behaviour is on the edge of the technical possibilities of applied and available instrumentation. I really appreciate and value the effort invested in this research. Ms. Kuznetsova in the first chapter introduces the topic well and stimulated my interest. I must confess, though, that with no a prior knowledge of the carotenoids, I would appreciate more detailed description and possibly supported by few more schemes and figures. That is also true for the chapter 1.7 where spectroscopic techniques are discussed. Need for **description of the techniques that were not use should** (had) be better **justified** or avoided. I consider the content of chapter 2.1 and 2.2 belonging to the general chemists' knowledge and not really needed in this thesis; instead I would prefer broader description of techniques covered in the chapter 2.3 and 2.4.

The **questions** posed at the end of the chapter 1.1 are indeed answered by the papers, however I would appreciate if the chapter 7 provides the **answers and overall conclusion in more broader range** and not only reviews of the individual papers. Also, inspired by the leading scientific journals, I would like to see the **description of authors contribution to the work.** Somehow more individualized approach to the thesis may also enhance its readability.

I can conclude that the scientific level of the four presented papers corresponds well to the doctoral work and therefore I **recommend the theses for the defence.**

Specific comments and questions. Are meant to offer my photochemistry view to the part of the science where I am a layman and possibly to extent the authors horizons. They are results of my curiosity and wish to enhance the scientific discussion in a positive way.

p. 30 The diode array spectrophotometer is used in the work. I should warn from using it for sensitive photochemical systems as the radiation power of the lamps are often not negligible. We have observed the isomerization of azobenzene during one second measurements. Did you check the stability of your compounds after multiple irradiation by the UV-Vis lamps?

Ch. 3 I was a bit puzzled by the paper's title, at first. Could the title also read: "Excited-State Dynamics of Fucoxanthin Isomers"? Accordingly, can you try to draw an appropriate part of the potential energy surfaces (possibly a correlation diagram) that would characterize the discussed photophysics? I am inspired by Figure 7 in the reference (Born, Fischer et al. 2007).

Paper 1, Figure 2. What is the origin of the "cis" peak in the spectrum of all-trans-fucoxanthin? Does it belong to the cis isomer that is present in a small amount?

Not being an expert in the field of carotenoids, I dare to ask: What are the arguments for assigning the 610-650 nm peaks to ICT-S_N bands?

Can you explain what: "a blue form that exhibit weaker *coupling* between the S1 and ICT means? What is meant by *coupling*? Is there and equilibrium?

I propose an alternative explanation for the blue part of transient species (Fig 3). The transient spectra are influence by the ground state bleach. Is the S* really needed for the explanation? The loss of vibrational structure in transient (for methanol compared to hexane) mirrors the GS spectral structure in these two solvents.

The output of the global analysis are eigen vectors in 2D (time, wavelength). Can you, please, show an example of the eigen vectors and the resulted species spectra? I would think, these may be more informative than the reconstructed spectra in particular times (as given in S3, 4).

The transient absorption spectra, sometimes, shift in wavelengths during the lifetime of the species. Then, neither single kinetic fit nor the global fit can help with the interpretation. Is it possible, that the difference in the behaviour of the described "blue and red" part of S1-ICT is caused by such shift? What would be the reason for the rocking of the spectra? Are you aware of the proper mathematical treatment of this behaviour or can you suggest it?

Ch.4 What do you understand under the term 0-0 band, please? Can you show it in the picture? I would not agree, that S2 energy (shown in Figure 4.2, p. 87) remains unchanged in various solvents.

Ch. 6 SI The figures show *deconvolution* of the spectra. Was really deconvolution technique applied or is it rather peak fitting? (Deconvolution is mathematical narrowing of the band widths while keeping band areas constant. (Antonov and Nedeltcheva 2000))

The physical quantities should be given as the value and its uncertainty. No uncertainty was calculated for the papers!

Key questions.

1. Which part of your work do you value the most? On the other hand, is there any place in your work, where you would reconsider the original (published) interpretation?
2. What, according to your opinion, is the most important question to be answered in the field of carotenoid chemistry?

Minor comments and questions.

Text shows quite a few signs of haste and glitches, some caused by copy-past techniques of composing the text.

p. 1 In the preface, we learn that only small part of the sunlight reaching the Earth's surface is being converted to electricity or heat. It is being estimated that natural photosynthesis utilizes 2.4 % of incident radiation. (Klán and Wirz 2009) What is then happening with the major portion of the light?

p. 6 Rotation around the C2 is described to give "identical of the original structure" whereas reflection in the plane "leaves the object in the same orientation". Is there a difference in the outputs of the operations?

p. 7 "A and B refer to the states that are, respectively, symmetric and *asymmetric* ...." Should read "antisymmetric".

p. 8 Figure 1-3. "The dotted red arrows show two processes – internal conversion (IC) and *energy dissipation*." Energy dissipation should be better called *vibrational relaxation*. I do not like showing these processes at once as one easily oversees that IC is an isoenergetic transition.

Figure 1-4a does not specify the solvent;

p. 31 "Extinction coefficient or molar absorptivity $\epsilon(\lambda)$" – is according to IUPAC called Molar absorption coefficient.

p. 31 The spectra are described as: "absorbance plotted versus certain wavelength", should read: absorbance versus range of wavelengths;

p. 167 In this *summary*, I would like to give a short *summary* ....

V Brně dne 5. 9. 2017

Doc. Dominik Heger, Ph. D.
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Cited literature

Antonov, L. and D. Nedeltcheva (2000). "Resolution of overlapping UV–Vis absorption bands and quantitative analysis." Chemical Society Reviews **29**(3): 217-227.

Born, R., W. Fischer, et al. (2007). "Photochromism of phenoxynaphthacenequinones: diabatic or adiabatic phenyl group transfer?" Photochemical & Photobiological Sciences **6**(5): 552-559.

Klán, P. and J. Wirz (2009). Photochemistry of Organic Compounds: From Concepts to Practice. Chichester, West Sussex, U.K., Wiley.

CHARLES UNIVERSITY PRAGUE
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Prague, September 5, 2017

Subject: Report on the Ph.D. thesis of Valentina Kuznetsova

Despite its rather general title, the doctoral thesis of M. Sc. Valentyna Kuznetsova "Time-resolved spectroscopy of light-induced processes" supervised by Mgr. Marcel Fuciman, Ph.D. from Faculty of Science, University of South Bohemia, České Budějovice focuses on application of time-resolved optical spectroscopy to investigations properties of carotenoids, mainly those with carbonyl groups, in solutions and in protein environment. It is based on four scientific papers published in respected journals. Valentyna Kuznetsova is the first author of two of them and the second author of one of them. Her contribution to each of the papers is specified in the front matter of the thesis.

The first chapter briefly introduces the role of carotenoids in photosynthesis and brings a detailed view of their electronic transitions and excited states. Finally, it describes the water-soluble orange and red carotenoid proteins which binds carotenoids and take place in photoprotection of cyanobacteria. The second chapter of the thesis starts with description of the reverse-phase HPLC system utilized for separation of the investigated carotenoids. It covers the basics of the femtosecond transient absorption spectroscopy together with details of the experimental set-up. Finally, the methods utilized for analysis of the spectrally and temporally resolved data are presented.

The next part of the thesis contains four chapters corresponding to the four published papers. These chapters are just slightly modified texts of the papers which is in my opinion one of the weaknesses of the thesis. In particular, the separation of some data to the "Supporting Information" instead of putting them to the right place in the main text (which is understandable in the case of the limited space of the journal paper), makes the reading of the thesis unnecessarily complicated. Moreover, the proper care was not taken to adapt the references to particular figures and tables to the thesis style (*e. g.* page 64: Fig. 3a should read Fig. 3-3a; page 55: Table 2 should read probably Table 3-2; page 97, Table 4-2, second line: Error! Bookmark not defined; page 117 Table 1 should read Table 5-1 and the references to figures 4 and 4a under the table should read 5-4 and 5-4a respectively; page 124 reference [13] should be in the superscript). The inclusion of the abstracts to each of the chapters seems redundant to me. Moreover, the key points of the individual chapters are summarized again in the last chapter.

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The last chapter summarizes the main results of the individual papers in a clear and concise way. However, presenting some links between results from the individual papers stressing out the general properties of the studied molecules would substantially improve this part of the thesis.

I have following questions to the thesis:

1. The time constants obtained by fitting lack the values of experimental uncertainties in the thesis, which makes hard for a reader to decide, where the differences are really significant. What are the uncertainties of time constants for instance in tables 3-2, 4-2 and 5-1? Similar objection apply also to the values of effective conjugation lengths in Chapter 4 and values of anisotropy and dipole moments displacements in Chapter 3.
2. Section 4.4. Discussion, p. 94: There is a discrepancy between N_{eff} values for Ifx-l in the text (8.5) and in the Fig. 4-7 (8.4). What is the right one?

Besides the questions, I have also minor remarks:

- Page 11, 2nd paragraph. "...in polar solvents the S_1 state lifetime is more than an order of magnitude shorter than in non-polar solvents. For example, the S_1 state lifetime of fucoxanthin is 20 ps in polar (methanol) versus 56 ps in non-polar solvents (n-hexane) ..." The difference doesn't seem to me as more than an order of magnitude.
- Page 13, 1st paragraph: The absorbance of blue-green light ...should probably read The absorption of ...
- Page 48, last paragraph: The excitation power of the pump pulses was 3–15 $\times$ 10^{13}, I guess.
- Page 58: density fumctional should read density functional
- Page 114: "The probe beam was further split into the probe ...a reference ..." should read probe ...and reference

Despite my critical remarks, the thesis brings important and valuable scientific data and presents them in a clear way. The work fulfills the requirements for doctoral thesis and can be accepted for the defense. In my opinion, Valentyna Kuznetsova presented very good work, prooved to be capable of scientific work, and deserves to be awarded the Ph.D. title.

doc. RNDr. Roman Dědic, Ph.D.

LUND
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DIVISION OF CHEMICAL PHYSICS

Report on the PhD thesis titled "Time-Resolved Spectroscopy of Light-Induced Processes" by Valentyna Kuznetsova

Valentyna's thesis is based on four papers, all of which are already published in the acknowledged international journals. The introduction section is well structured and thesis is written in a clear language, even the very complex discussions in Paper IV.
The main theme of the thesis is analysis of photophysical properties of carotenoids both in solutions and in proteins. It seem that the more carotenoids are studied the more complex picture of their properties is revealed. This is plainly demonstrated in Valentyna's thesis, where multiple approaches were used to unravel carotenoid properties. These approaches include studying different isomers of the carotenoids, comparing properties of these molecules in solvents with varying polarity and proticity and comparing spectroscopic properties of carotenoids imbedded in proteins. While reading I especially appreciated an elegant way, how the unknown carotenoid from Chromera velia was characterized in a comprehensive experimental and theoretical study, presented in paper II, by comparing the properties of this carotenoid to other similar molecules (even though I think that the lactone ring will be eventually found in this carotenoid once its structure is determined).

Overall, I believe this thesis fulfills the requirements of the PhD work should be accepted for the defense.

Some questions and comments to the candidate.

1. Since the student is expected to defend her thesis, I would like to ask a question regarding contributions. I noticed that your contribution to the paper II is rather minor. I was wondering if you could elaborate on your contribution to justify the inclusion of this paper in your thesis?

Introduction section

2. There is quite a bit mentioning of the loss of vibronic structure in the absorption of some carotenoids under certain conditions. Could you provide more explanation what is the mechanism of this? For example why vibronic structure is lost in the case of the carbonyl carotenoids in the polar solvents?
3. There is a statement that dependence of the S_2 state lifetime on the conjugation length is not straightforward. Can you elaborate why and explain what are the possible deexcitation mechanisms of the S_2 state?

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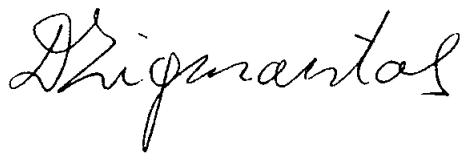
Experimental methods

4. The sapphire plate is used for the generation of the probe white light. Can you tell a bit more about this: what properties sapphire plate has, what is the main mechanism of white-light continuum generation, and what about polarization?

Research section

5. A general question regarding all of the EADS analysis. The short time component in EADS spectra looks very different in different measurements of different molecules and its lifetime ranges from 40 to 300 fs. It is mostly associated with the S_2 state, however, since it can take very different forms, there must be more contributions to this component. Can you comment on that?
6. Figure 3-2. In the spectrum of all-*trans* isomer of fucoxanthin, there is some small structured absorption in the 300 – 350 nm range. Does this mean that there is still some *cis* isomers remaining in the sample?
7. On page 115 you mention that the energy separation between vibronic peaks varies from 1350 cm^{-1} (lycopene) to 1460 cm^{-1} (neurosporene). There should be more or less same vibrations in all carotenoids in this spectral region, then why frequencies are so different?
8. You claim that "excess vibration energy in the S_2 state further increases the conformation disorder in the S_1 state" (page 116) and "generates excess vibrational energy in the S_1 state " (page 121). Could you elaborate why and how?
9. On page 165 the red band is extracted from absorption fits in Figure S6-1b,c. It does not look very much like carotenoid absorption, then what is the origin of this band?

Sincerely yours,
Donatas Zigmantas



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