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Ph.D. thesis

Excited state dynamics of carotenoids in solution and proteins

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Dedicated to Lidu and Josef

Annotation

Time resolved spectroscopy is one of the crucial methods used to study processes on molecular level in biological systems. It is useful especially for monitoring fast processes that take a place in photosynthetic apparatus of photosynthetic organisms, such as electron and energy transfer. The integral parts of photosynthetic apparatus are carotenoids, whose role in the photosynthetic apparatus is not as well explored as it is for chlorophylls. It was proved that carotenoids actively participate in energy transfer processes in photosynthetic antennas. They have a crucial role in protection against excess energy damage. They are also electron donors in both antennas and reaction centers. The fact that photo-physical properties of carotenoids are much different from properties of others organic pigments, complicates studies of their functions in photosynthesis as well as in other biological systems. This thesis employs advanced methods of femtosecond spectroscopy to obtain more information about carotenoid functions in some biological systems and in solution with special focus on carotenoids containing carbonyl group**.**

Anotace

Časově rozlišená spektroskopie je jednou z klíčových metod používaných ke studiu procesů odehrávajících se na molekulární úrovni v biologických systémech. Její uplatnění je neocenitelné zejména při sledování rychlých procesů probíhajících ve fotosyntetickém aparátu fotosyntetizujících organismů, jako přenos energie či elektronu. Tato metoda je s úspěchem využívána ke studiu procesů přenosu energie a elektronu. Nedílnou součástí fotosyntetického aparátu jsou karotenoidy, jejichž role ve fotosyntetickém procesu je výrazně méně prozkoumána než v případě chlorofylů. Bylo prokázáno, že karotenoidy se aktivně účastní přenosu excitační energie v anténách a hrají klíčovou roli v ochraně fotosyntetických organismů proti poškození v důsledku nadměrného ozáření. V neposlední řadě působí rovněž jako donory elektronu jak v anténách tak v reakčních centrech. Fotofyzikální vlastnosti karotenoidů jsou však výrazně odlišné od vlastností jiných organických barviv a tato skutečnost značně komplikuje studium jejich funkcí jak ve fotosyntéze tak i v jiných biologických systémech. Tato práce se zabývá využitím pokročilých metod femtosekundové spektroskopie k prohloubení znalostí funkcí karotenoidů v různých biologických systémech, se speciálním zaměřením na karotenoidy obsahující karbonylovou skupinu.

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V Nových Hradech, 1. dubna 2010

Pavel Chábera

List of papers

I. **Effect of carotenoid structure on excited-state dynamics of carbonyl carotenoids,** P. Chábera, M. Fuciman, P. Hříbek and T. Polívka, *Phys. Chem. Chem. Phys*., **2009**, 11, 8795-8803

II. **Ultrafast dynamics of hydrophilic carbonyl carotenoids - relation between structure and excited state properties in polar solvents**, P. Chábera, M. Fuciman, K. R. Naqvi and T. Polívka, *Chem Phys.,* **2010**, **doi**: 10.1016/ j.chemphys.2010.01.007

III. **Excited state properties of aryl carotenoids,** M. Fuciman. P. Chábera, A. Župčanová, P. Hříbek, J. B. Arellano, F. Vácha, J. Pšenčík and T. Polívka, *Phys. Chem. Chem. Phys*., **2010**, 12, 3112-3120

IV. **Optimal Control of Peridinin Excited-State Dynamics,** B. Dietzek, P. Chábera, R. Hanf, S. Tschierlei, J. Popp, T. Pascher, A. Yartsev and T. Polívka, *Chem. Phys.*, in press

V. **Femtosecond Carotenoid to Retinal Energy Transfer in Xanthorhodopsin**, T. Polívka, S. P. Balashov, P. Chábera, E. S. Imasheva, A. Yartsev, V. Sundström and J. K. Lanyi, *Biophys. J.,* **2009**, 96, 2268-2277

VI. **Excited-state properties of the 16 kDa red carotenoid protein from *Arthrospira maxima***, P. Chábera, M. Durchan, C. A. Kerfeld and T. Polívka, *manuscript*

My contribution to the papers

In **papers I, II** and **IV** I was involved in the experimental planning as well as in the significant part of the experimental work and data analysis. I wrote **papers I** and **II,** and was also involved in writing part of paper **IV**. In **papers III** and **V** I did substantial part of the experimental work and part of the data analysis. In **paper VI** I did all experimental work, part of data analysis and writing.

On behalf of the co-authors, the above mentioned declaration was confirmed by

Prof. RNDr. Tomáš Polívka, Ph.D.
(supervisor and co-author of the papers)

Papers not included in thesis

VII. **Four-Wave-Mixing Spectroscopy of Peridinin in Solution and in Peridinin-Chlorophyll-a Protein**, N. Christensson, P. Chábera, R. G. Hiller, T. Pullerits and T. Polívka *Chem. Phys,* **2010, doi**: 10.1016/ j.chemphys.2009.12.011

VIII. **β-carotene to bacteriochlorophyll c energy transfer in self-assembled aggregates mimicking chlorosomes**, J. Alster, T. Polívka, J. B. Arellano, P. Chábera, F. Vácha and J. Pšenčík, *Chem, Phys*., **2010, doi**: 10.1016/ j.chemphys.2010.02.006

IX. **Ultrafast Intramolecular Charge Transfer in Tetrapyrazino-porphyrazines Controls the Quantum Yields of Fluorescence and Singlet Oxygen**, V. Nováková, P. Zimčík, M. Miletín, L. Váchová, K. Kopecký, K. Lang, P. Chábera and T. Polívka, *Phys. Chem. Chem. Phys.,* **2010**, 12, 2555-2563

Contents

Acknowledgements

1. General Introduction

Our planet obtains over 170 PW (petawatt, 10^{15} W) of solar energy at the upper atmosphere. Approximately 30% is reflected or scattered back to space, resulting in about 120 PW (120 x 10^{15} W) of solar energy penetrating to the Earth surface. That is 120 PJ (120 x 10^{15} J) per second! Thus, the amount of solar energy hitting our planet in one hour is comparable to the annual energy demand of the whole mankind (assuming the current consumption of 14 TW per year). The Earth receives so excessive amounts of solar energy that in half of a year it gets about the same amount as could be possibly retrieved from Earth's non-renewable resources together if treated as nowadays.

To use this enormous amount of energy to power the human society is tempting. In principle, all we need to exploit this generous offer given from the Sun is to develop a mechanism, which would be able efficiently harness the solar energy. Yet, years of research have proven this task to be extremely difficult. On the other hand, the entire world of photosynthetic organisms has already succeeded in this task. The eons of evolution of photosynthetic organisms generated a broad variety of mechanisms how to collect sunlight and transform its energy into other forms of energy that could be directly used by photosynthetic organisms. Besides photosynthesis, a variety of light-driven processes occurs in Nature, and detailed understanding of these processes is the key to mimicking natural processes in artificial devices that would be in future used to harness the solar energy for the needs of mankind.

1.1. Light driven processes

There is a large group of light-dependent processes in nature, and life, as it is known nowadays, would not be possible without light.

A typical light-driven process is vision. The vast majority of animals, including humans, obtain a substantial part of information about their surrounding by the visual perception, in which incident photons are transformed into chemical signal via a cascade of processes. In human eye, photons are absorbed by rods or cones, the photosensitive receptors consisting of transmembrane protein opsin and chromophore retinal.[1] Except vision, many organisms utilize various types of light-sensitive proteins to detect light intensity that is of enormous importance for circadian rhythms that allows organisms to follow the 24 hours day cycle[2]. A number of other examples, such as phototropins of plants, which control a range of physiological responses, including phototropism, light-directed chloroplast movement, and light induced stomatal opening[3], can be found. Similarly, phototropism in bacteria is achieved via light receptors called BLUF domains[4].

Since all these processes are triggered by light absorption that results in excitation of the primary pigment, exploring the excited-state dynamics following the excitation is crucial for understanding of the light-driven processes in nature. This is of course also valid for probably the most important light-driven process on the Earth, photosynthesis.

Photosynthesis is an ingenious biological process by which the solar energy is captured and consequently stored by a series of events converting the photon energy into chemical energy, which is necessary to power the Earth's life. Plants, algae and a variety of bacteria[5] utilize sunlight to run their cellular processes and they produce most or all of their biomass through chemical reactions which are driven by light. Thus, a large fraction of Earth's energy resources results from photosynthetic activity in either recent (biomass production and the consecutive food chain) or ancient times (fossil fuels). The primary steps in transformation of solar radiation into chemical energy via the photosynthetic process take place in pigment-protein complexes known as reaction centers (RC).[6] Release of an electron from the lowest excited singlet state of (B)Chl special pair leads to formation of a radical pair that is stabilized by subsequent electron-transfer reactions, thus providing the driving force for metabolic processes. Because photosynthetic organisms habituate under various light conditions, suitable adaptation mechanisms are required to utilize given resources as much as possible. This important goal has been achieved by development of another type of pigment-protein complexes, the photosynthetic antennas[7], which simultaneously fulfill two functions. First, they provide light harvesting at limiting photon densities through efficient funneling of excitation energy to the photoactive pigments in RC. Second, they afford protection against photodamage under high-light conditions by providing the dissipative channels for radiationless decay of superfluous energy. The antennas allow great increase of the absorption cross section by employing another type of light-harvesting pigments, carotenoids. Incorporating of carotenoids into antenna complexes has enabled harvesting photons even in the spectral regions where the chlorophyll absorption is weak. In antenna complexes, pigment-pigment and pigment-protein interactions are crucial to achieve efficient excitation energy transfer from the peripheral light-harvesting antennas to the photosynthetic reaction centers.[8]

Many fundamental questions relating the mechanism of energy storage by natural photosynthesis remains unsolved. A number of these questions are directly related to the effort to develop artificial photochemically based systems for solar energy storage, in which water is oxidized to molecular oxygen, along with either hydrogen production or CO_2 reduction – to creation of the artificial photosynthesis. Studying details of excited-state processes of molecules

participating in light-harvesting such as carotenoids is thus important way how to progress in this effort.

2. Carotenoids

Carotenoids represent a large family of natural pigments and together with chlorophylls are the most abundant pigments in nature. There are over thousand of carotenoids known today and while they are perhaps best known for their bright colors, they have well documented multiple functions in nature (for overview see Ref. [9]).They can be found in most living organisms such as bacteria, fungi, algae, green plants, animals and humans. However, only microorganisms and plants employ metabolic pathways for carotenoid synthesis. Thus the only way how to supply animals (including humans) by the vitally important carotenoids is via their diet. Carotenoids are found in almost every photosynthetic apparatus serving as accessory light-harvesting pigments; they efficiently absorb light in the 460 – 550 nm region, where the light-harvesting capacity of chlorophylls is poor. Simultaneously, carotenoids protects the photosynthetic apparatus against photodamage by quenching both singlet and triplet states of (bacterio)chlorophyls.[10,11]

Besides photosynthesis, carotenoids also have important function for structural integrity and stability. For example, carotenoids are crucial for protein folding in certain proteins; without carotenoids tertiary structure of the major antenna complex of plants, LHCII, cannot be established properly, resulting in protein malfunction. Carotenoids are also known as efficient quenchers of dangerous singlet oxygen and various reactive radicals by intercepting the chain of oxidative reactions.[11] In recent years, synthetic carotenoids have become popular food colorants and food supplements (antioxidants) as they are involved in the human body protection mechanisms against various diseases including lung, breast and skin cancer, macular degeneration, arteriosclerosis and others.[12,13]

2.1. Structure of carotenoids

The versatility of carotenoids is unmatched by any other natural pigments and is directly related to their unique spectroscopic properties resulting from a structure of carotenoid molecule, derived from a 40-carbon polyene chain, which forms a conjugated π-electron system responsible for most of the spectroscopic properties of carotenoids. This chain is often terminated by cyclic end-groups (rings) and can be complemented with oxygen-containing functional groups (see **Fig 1**). Hydrocarbon carotenoids are known as carotenes, while their oxygenated derivatives are called xanthophylls. β-carotene, the principal carotenoid in carrots, is a typical carotene. Lutein, the yellow pigment found in egg yolk and the pigment responsible for the yellow color of human macula, is a common

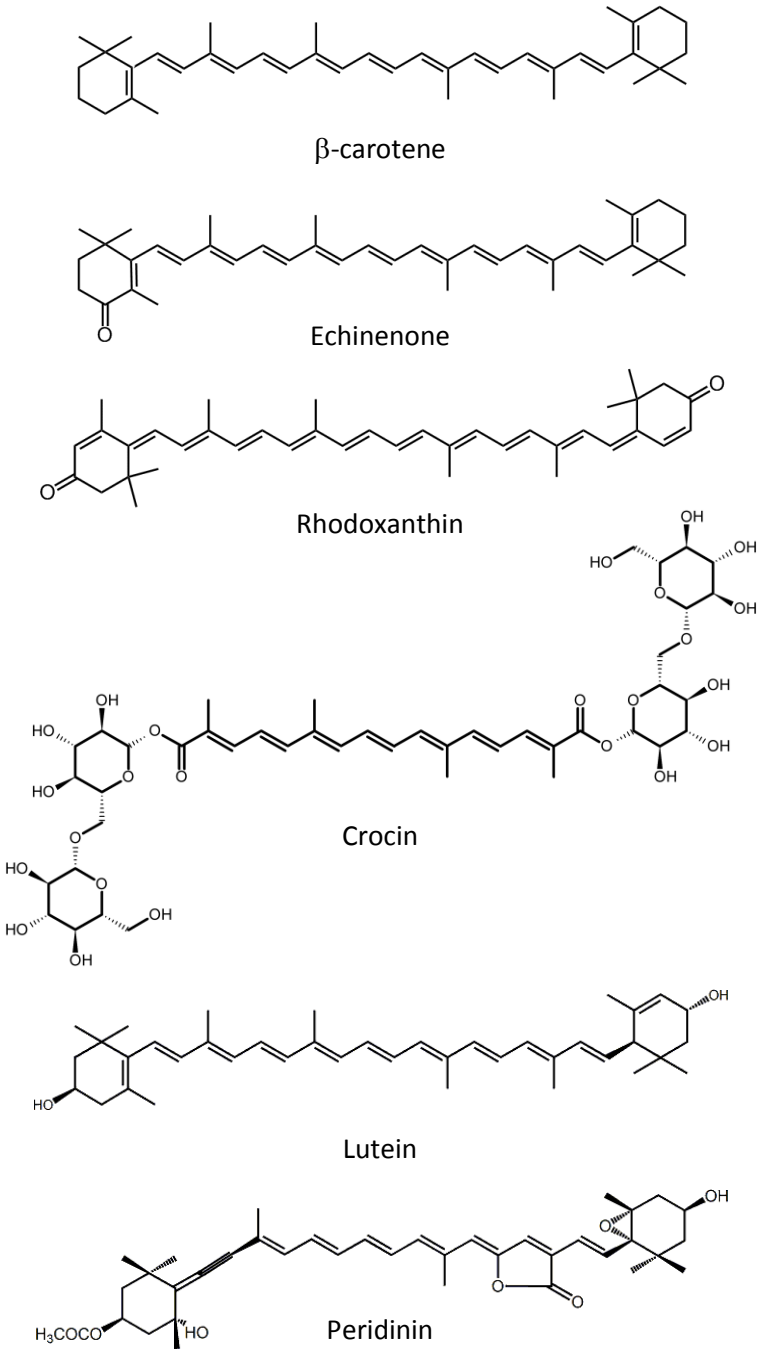

Fig. 1 *Molecular structure of several carotenoids discussed in thesis. See text for more details*

xanthophyll. Other examples of variations in carotenoid structure are demonstrated in **Fig 1**, showing molecular structures of a few carotenoids, which are further discussed in this thesis and their structure is in more detail described below.

Echinenone, a carotenoid present in photosynthetic proteins of algae and cyanobacteria has molecular structure similar to β-carotene, but one of its terminal rings is further functionalized by a conjugated carbonyl group (C=O). The presence of carbonyl group within the conjugated chain is known to alter the photo-physical properties of carotenoids, making them sensitive to the solvent polarity (see chapter **2.4** and Ref.[14]). This effect is especially considerable in peridinin, a highly functionalized carotenoid with carbonyl group attached asymmetrically via the lactone ring.[15] While in some carotenoids the conjugated backbone remains unsubstituted, examples of extreme substitutions can be also found in nature. Typical example is crocin, carotenoid occurring in high concentrations in carpels of the crocus flower (*Crocus longiflorus*), and is responsible for the red-gold color of saffron. The bulky end groups of crocin contain a large number of hydroxyl groups that makes crocin water-soluble, contrary to the vast majority of natural carotenoids that are strictly hydrophobic.[16] Another specific structural feature of carotenoids is demonstrated in rhodoxanthin, a member of the retro-carotenoid family. These carotenoids have all single and double bonds of the conjugated polyene system shifted by one position[17], which in the case of rhodoxanthin results in the conjugated carbonyl groups positioned in *s-trans* orientation with respect to the main conjugated backbone. This feature has also a significant impact on its photo-physical properties.[18]

2.2. Excited states of carotenoids

Even though the structure of carotenoids is highly versatile, there is a characteristic pattern repeating in all carotenoids. It is the carbon-carbon chain consisting of alternating single and double bonds that forms a conjugated system of π-electrons. The conjugated system makes the carotenoids naturally hydrophobic (unless they are substituted with hydrophilic groups), and it is responsible for their unique spectroscopic properties. A lot of effort has been put into detailed understanding of excited-state properties of carotenoids in the past decade (for reviews see Refs. 19-21). The structure of conjugated chain assorts carotenoids to the C_{2h} symmetry resulting in a set of states with A_g^- and B_u^+ symmetries in the idealized C_{2h} point group (**Fig 2**). The peculiarity of carotenoids is given by the fact that due to strong electron correlation, the second A_g^- state

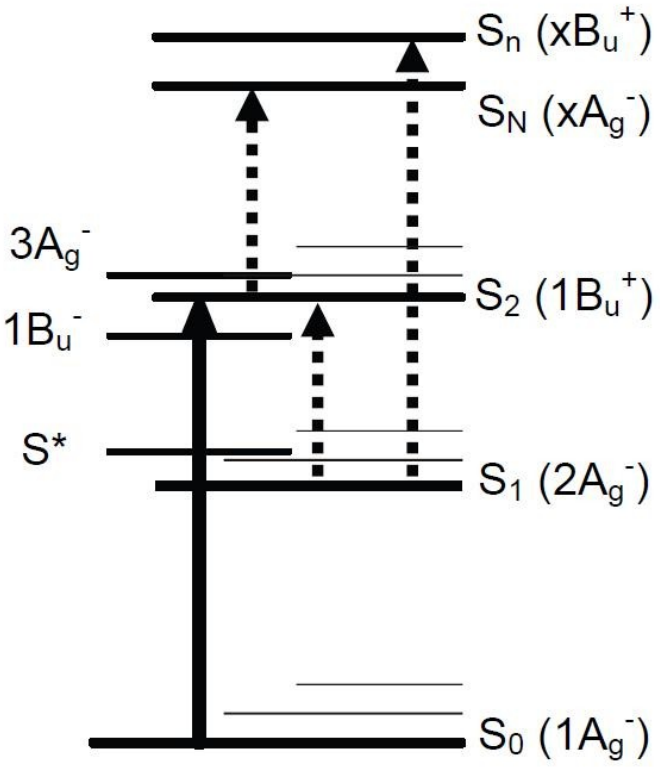

Fig. 2 *Simplified energy level scheme of a carotenoid molecule. The solid arrow represents the S_0–S_2 transition; the dotted arrows are transitions corresponding to transient signals occurring after excitation.*

($2A_g^-$) is pushed below the lowest excited state with B_u^+ symmetry ($1B_u^+$). Since the ground state is of A_g^- symmetry ($1A_g^-$), this results in a situation where the lowest excited state has the same symmetry as the ground state (A_g^-). Because transitions between states with the same symmetry are forbidden for one-photon processes, the lowest excited state becomes optically inaccessible, and it is often referred to as a "dark state". On the other hand, the allowed transition between the ground state ($1A_g^-$) and the first excited state with B_u^+ symmetry ($1B_u^+$) is responsible for the strong absorption of carotenoids in the blue-green region, resulting in characteristic colors of carotenoids (the solid arrow in **Fig 2**). These three states are usually called S_0 ($1A_g^-$), S_1 ($2A_g^-$) and S_2 ($1B_u^+$), and this notation is also used throughout the following chapters.

When the carotenoid molecule is excited by absorption of a photon via the S_0-S_2 transition, it undergoes a complex relaxation dynamics and descends to the S_1 state within a few hundred femtoseconds. The radiationless relaxation continues further from the S_1 state to the ground state on a picoseconds time scale, with the actual S_1 lifetime depending on the conjugation length of the particular carotenoid (*N*), ranging for natural carotenoids from 1.5 ps for the longest to ~300 ps for the shortest.[19] These relaxation processes are in the scientific interest of plenty of studies and experimental approaches. In this thesis we applied technique called time resolved spectroscopy, where after excitation by a short laser pulse (pump), another short pulse (probe) is sent to the excited sample with controlled time delay (this experimental technique is elaborated in chapter 3.2.). When the carotenoid molecule is excited, new excitation possibilities are introduced and photons with different energies can be absorbed. Such a process of exciting already excited molecule by populating higher-lying states (dotted arrows in **Fig 2**) is called excited state absorption (ESA). In carotenoids, the most common ESA occurs from the S_1 state (**Fig 2**), populated by internal conversion from the S_2 state, to a high-energy state of B_u^+ symmetry usually denoted as S_n. If the S_1 state is populated, the incident photon with energy equal to the S_1-S_n transition can be absorbed. This results in specific spectroscopic signature in transient absorption spectra, the S_1-S_n ESA band, whose time evolution reflects relaxation dynamics of the S_1 state.

Moreover, a lot of results obtained during past few years show that, except the three states described above, there are several additional dark states that may exist within the S_1-S_2 energy gap. These states were suggested to be important for carotenoid photophysics (**Fig 2**), but details of their participation in relaxation processes remain a matter of considerable debate [20-24] due to several principal difficulties. For example, spectroscopic markers of the so-called S*state[22] and ICT state[19,25] are readily observed in various experiments, but these states have never been predicted by calculations (the S* and ICT state will be discussed in more detail in the following chapters). On the other hand, an excited state having B_u^- symmetry (1 B_u^-) was predicted to be within the S_1-S_2 gap for carotenoids with $N >$ 10 on the basis of quantum chemical calculations,[26] but no clear spectroscopic evidence of this state has been provided so far. Thus, its exact role and conditions of appearance in the carotenoid excited state manifold are still unclear.[20,21,24] For detailed review summarizing the quest for identification of the carotenoids dark states see Ref.21.

2.3. The S* state

A few years ago Gradinaru et al. observed in the S_1-S_n ESA band (S_1-S_n band) of spirilloxanthin, a long carotenoid having linear conjugated chain consisting of 13 conjugated C=C bonds, peaking at ~590 nm a distinct shoulder at ~540 nm. Interestingly, the lifetime of this spectral feature was significantly longer than the S_1 lifetime (6 ps vs. 1.4 ps).[22] To explain this observation, these authors proposed existence of a new state in the spirilloxanthin excited state manifold, the S* state. Nowadays, the S* state footprints are firmly observed in carotenoids with long conjugation as a long-lived distinct shoulder in the red part of the S_1-S_n band.[18,22,27] Since no direct connection between the S_1 and S* states was observed in earlier studies, both states were assumed to be two energetically close states decaying independently to the ground state.[19] Nevertheless, a few recent studies[28,29] showed that a certain fraction of the S* population may decay to the S_1 state, placing the S* above the S_1 state as a separated electronic state. Consequently, on the basis of recent studies, the S* was suggested to be directly associated with a twisted conformation of the carotenoid molecule in the excited state.[29,30,31] This conclusion is however contradicted by the hypothesis assigning the S* state to a hot ground state ("hot S_0"), populated by impulsive Raman scattering.[32,33] It is worth noting that the "hot S_0" hypothesis cannot explain the already mentioned observations of S*-S_1 transfer (Refs. 28 and 29), and it was also challenged by a recent study employing 2D electronic spectroscopy on β-carotene, disclaiming the possibility that the observed signals could originate from a hot ground-state.[34]

As it is apparent from the description above, detailed description of the S* state is still missing. Since the S* state is (in many cases) inseparable part of excited state dynamics, a deeper knowledge about its origin, energy and involvement in relaxation processes is needed. Results presented in this thesis shed more light on this topic (**papers I** and **III**).

2.4. Carbonyl carotenoids

Carbonyl carotenoids form a special group of xanthophylls with a carbonyl group (C=O) incorporated in the conjugated system (see **Fig 1**). They are the most common carotenoids, spanning a broad field of functions. Perhaps the most studied function of carbonyl carotenoids is light-harvesting in the light harvesting antennas of marine dinoflagellate *Amphidinium carterae*,[35,36] in which carotenoid to chlorophyll-*a* energy transfer efficiency approaches nearly 100 %.[35-38] It is worth noting that carbonyl carotenoids have also been successfully applied in assembling of artificial light-harvesting systems, in which their specific properties allowed to tune energy transfer efficiency by solvent polarity.[39]

The most important effect of introduction the carbonyl group into the conjugated system is that it significantly changes excited state properties of the particular carotenoid.[14,15] First, the presence of a carbonyl group considerably narrows the S_1–S_2 gap.[15] This has an important impact on functionality of these carotenoids in light-harvesting systems of marine organisms. The reduction of the S_1–S_2 gap is manifested by shift of the S_2 state towards lower energies while the S_1 energy remains (nearly) unchanged.[15] Consequently, absorption band of carbonyl carotenoids is red-shifted, allowing more effective capturing of green light, which penetrates deepest in the water masses. Simultaneously, energy of the S_1 state is still high enough to mediate energy transfer to chlorophyll-*a*.

Introducing the carbonyl group into the carotenoid conjugated system also induces several polarity-dependent effects. Influence of the carbonyl group is well demonstrated by an asymmetric broadening of the absorption spectrum accompanied by a loss of vibrational structure caused by broader distribution of various ground-state conformers when carbonyl carotenoid is dissolved in polar solvent.[40] Perhaps the most important polarity induced change in carbonyl carotenoids is shortening of the S_1 lifetime. Peridinin, a flagship among carbonyl carotenoids, has the S_1 lifetime in nonpolar solvent (*n*-hexane) as long as 160 ps, but it is shortened to ~9 ps in polar solvents such as methanol or acetonitrile.[14]

The lifetime shortening and other polarity-induced effects are usually accompanied by appearance of new ESA band in transient absorption spectra, which is present due to population of an intramolecular charge transfer state (ICT) within the excited state manifold of carbonyl carotenoid as a consequence of the conjugated carbonyl group. [14,15,40,41]

2.5. The ICT state

Although questions concerning origin of the ICT state, its relation to the S_1 state, and the way how the ICT state is involved in the excited state processes of carbonyl carotenoids[40,42-44] remain unanswered, it is now well-accepted that the interaction of the ICT state with the S_1 state is the reason for the observed shortening of the S_1 lifetime. Appearance of new transient absorption band attributed to the ICT-$S_{n'}$ transition was then taken as a necessary condition associated with the polarity-induced shortening of the S_1 lifetime. Consequently, the intensity of the ICT band could be correlated with the magnitude of the S_1 lifetime shortening. However, this simple rule cannot be valid, because it obviously contradicts some experimental observations: the ICT band was observed in several cases (e.g. spheroidenone, Ref. 15) in the transient absorption spectra, but it was not accompanied by the S_1 lifetime shortening. Moreover,

opposite effect was observed recently in some hydrophilic carotenoids [18]); the S_1 lifetime of crocin measured in methanol reached 142 ps, it was shortened to ~59 ps in water, yet this change was not accompanied by appearance of the ICT band.

Until recently the lifetimes of the S_1 and ICT states were believed to be identical due to strong S_1-ICT coupling.[15] However, a next step further was recently done by Chatterjee et al, who were able to distinguish a separate lifetimes of the S_1 and ICT states using peridinin analogs.[45] Moreover, specific vibrational frequencies were recently assigned to either S_1 or ICT states of peridinin.[43,46] Thus, concluding this with previous investigations, the uprising model suggests that the S_1 and ICT states are likely two potential minima at the S_1 potential surface. The minima are separated by a barrier whose height is influenced by solvent polarity[15,44,45] and most likely also by the length of conjugated chain.[45]

Even though the ICT state attracted a lot of attention in the past decade, many questions regarding the appearance and character of the ICT bands or the essence of the ICT state itself remain unanswered. Conclusions presented in this thesis intend to answer some of these questions (**Paper II** and **V**).

2.6. Carotenoid – protein complexes

Binding of a chromophore in protein creating a functional pigment-protein complex is of vital importance in nature. For example, covalent binding of the photoactive chromophore retinal to the protein opsin in pigment-protein complex rhodopsin is crucial precursor in the first events of the light perception by the *vertebrae* eye. An important connection of pigments and proteins can be also found in nearly all photosynthetic organisms, serving as photosynthetic light harvesting antennas.[5] The widespread use of various photosynthetic antennas is common light-harvesting strategy of various photosynthetic organisms for increasing the efficiency of light energy utilization. The antennas are usually large multi-protein complexes containing a number of chromophores, namely (bacterio)chlorophylls and carotenoids. While in higher plants the carotenoids are typically used predominantly for protecting the photosynthetic apparatus against the excess light energy, in microorganisms is their primary role shifted more towards light-harvesting. There are even light-harvesting systems in which carotenoid is dominant light-harvesting pigment.[38]

However, not all pigments bound in protein are involved directly in light-harvesting, as it is in the case of orange carotenoid protein (OCP) that will be discussed in more detail in chapter **2.6.2.**

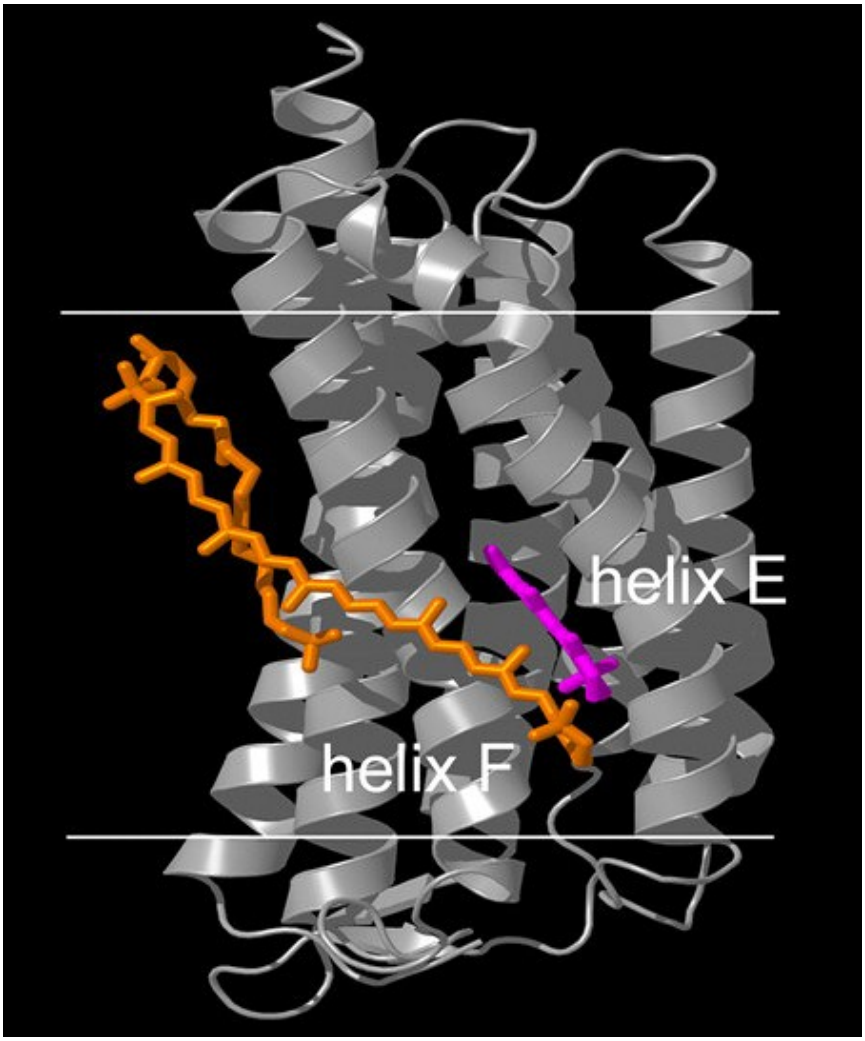

Fig 3. *Structure of xanthorhodopsin, showing the carotenoid salinixanthin (orange) and retinal (purple). Horizontal lines symbolize the membrane.*

2.6.1. Xanthorhodopsin

Although energy transfer between carotenoids to chlorophyll is common in photosynthesis, no specific antenna pigments were identified in retinal-based proteins serving as a light-driven transmembrane proton pump. However, in 2005 it was shown that even these systems may use light-harvesting antenna. Xanthorhodopsin,[47] protein complex similar to bacteriorhodopsin occuring in extremely halophilic bacterium *Salinibacter ruber*, utilizes carotenoid as antenna pigment A small (25 kDa) membrane protein binds two chromophores serving as donor and acceptor of energy. While the acceptor chromophore is retinal, the donor pigment is a carbonyl carotenoid salinixanthin serving as unique single-pigment antenna that enhances light-harvesting capability of the retinal. It was shown that energy captured by the salinixanthin is transferred to the retinal chromophore with efficiency of about 45 % from the short-lived S_2 state[48]. Therefore, there must be a short distance and favorable geometry between the chromophores in order to achieve such efficiency. This was recently confirmed by xanthorhodopsin structure that has been determined with 1.9 Å resolution.[49] **Fig. 3** shows xanthorhodopsin structure containing carotenoid (orange) and retinal (violet).

2.6.2. Orange carotenoid protein

Photoprotection mechanism known as non-photochemical quenching[50] (NPQ) is usually associated exclusively with plants. It is a regulatory process that is necessary to balance absorption and utilization of light energy for minimizing photo-damage under high light conditions. This is achieved by quenching the singlet excited states of chlorophylls and excess excitation energy is dissipated as heat. However, experiments in past few years showed that this comparable photoprotective mechanism operates also in cyanobacteria.[51] The excess energy absorbed by the phycobilisomes, cyanobacterial light harvesting pigment-protein complexes, is converted into heat by the NPQ mechanism, protecting the PSII reaction centers from photo-induced damage under superfluous light conditions. It was shown that the cyanobacterial water-soluble orange carotenoid binding protein (OCP) is essential for this photoprotective mechanism in cyanobacteria.[51] Although the OCP was first described by Wu and Krogman in 1997[52] the structure remained unknown until 2003 when the crystal structure of OCP isolated from the cyanobacterium *Arthrospira maxima* was resolved to 2.1 Å resolution.[53]

OCP, 35 kDa water soluble protein (**Fig 4**, left), consists of two protein domains. It contains a single pigment, the carbonyl carotenoid 3'-hydroxyechinenone (hECN) that is embedded in the protein, spanning both of the domains being docked in the binding pocket of C-terminal domain via its carbonyl

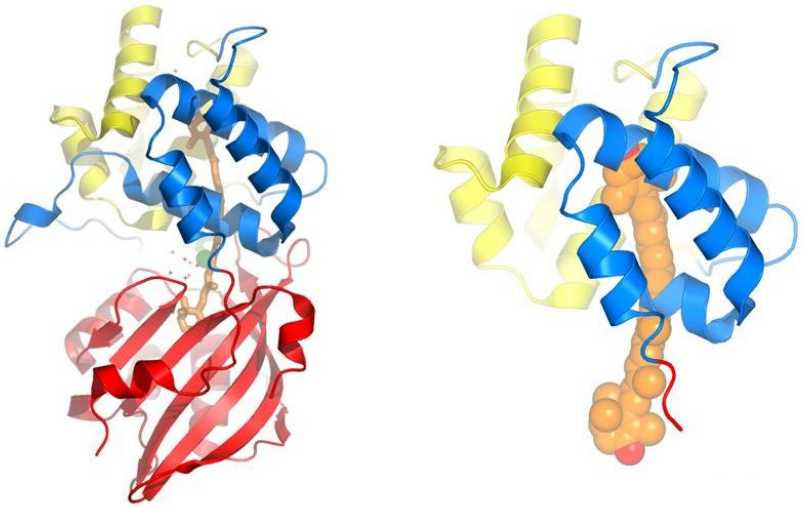

Fig 4. *Structure of OCP (left) and proposed model of RCP structure (right). In RCP the C-terminal domain is missed, thus protein lock is released and H-bonding lost.*

group (C=O).[53] This noncovalent interaction induces a twist of the hECN terminal ring containing the C=O group. This conformation change results in prolongation of the effective conjugation length of hECN, thus consequently in shortening of the carotenoid S_1 lifetime, which is ~6.5 ps in solution, while ~3.3 ps in the OCP.[54] Moreover, this conformational change of hECN result in stabilization of an intramolecular charge-transfer (ICT) state within the carotenoid excited state manifold. While no ICT bands are observed in hECN transient spectra in solution a strong ICT band appears at about 650 nm for hECN upon disturbing its innate symmetry when trapped in the OCP.[54]

There is another form of the OCP lacking the C-terminal domain, resulting in smaller 16 kDa protein with red-shifted absorption spectrum, called red-carotenoid protein[55] (**Fig 4**, right and **Paper VI**).

3. Experimental methods

When facing a scientific problem, not only properly formulated questions and solid theoretical background are necessary; the correct choice of experimental method is crucial as well. The following chapter describes the experimental approaches used while seeking for the answers to the questions described in this thesis by performing various experiments and by analysis of the gathered data.

3.1. Steady-state absorption spectroscopy

Absorption spectroscopy is very basic technique, but it is still indispensable for acquiring the fundamental information about spectroscopic properties of a studied system. In principle, absorption spectrum provides information about the amount of electromagnetic energy (typically in the visible region) absorbed by a studied system as a function of wavelength. If light with a broad spectrum, covering the whole visible spectral region, is directed to a bulk of molecules, they absorb photons with energies equal to the allowed one-photon transitions from the ground state. Certain wavelengths are then missing in the original light spectrum after passing through the sample. If the amount of absorbed light is plotted versus particular wavelengths, the sample-specific spectral pattern is created, typically called steady-state absorption spectrum. The spectral position of the absorption peak provides us direct information about energy of the absorbing state which is crucial essentially for all experiments described in this thesis. Furthermore, the shape of the absorption spectrum can provide deeper insight into the molecular steady-state properties such as the degree of isomerization, aggregation or photo-damage.

Apart from a few exceptions described in the respective papers, absorption spectra presented in this thesis were measured in a 1 mm quartz cuvette in a Perkin Elmer Lambda 35 absorption spectrometer with 0.5 nm resolution.

3.2. Transient absorption spectroscopy

Although the steady-state absorption spectroscopy is useful tool for investigating certain steady-state molecular properties, it is unable to reveal information about intrinsic dynamics of the studied system. Even though the fastest electronic devices such as streak cameras or avalanche photodiodes can currently follow processes taking place on picosecond or even several hundreds of femtoseconds time-scales, techniques with better time resolution are required. It

was proven that transient absorption spectroscopy is such an eligible experimental approach allowing monitoring directly molecular dynamics with sub-100 fs time resolution.[56]

3.2.1. General considerations

Among the ultrafast (femtosecond) spectroscopy experiments, the most used is likely the transient absorption or pump-probe technique. It is based on a laser delivering short (<100 fs) pulses whose output beam is split into two, forming the pump (excitation) and probe beam. The general scheme of pump-probe setup is in **Fig 5**. The excitation pulse triggers the process of interest, typically a photochemical reaction. In most experimental schemes there is a chopper blocking every second excitation pulse to allow comparison between the excited and non-excited sample. In most cases, when using probe in the visible spectral

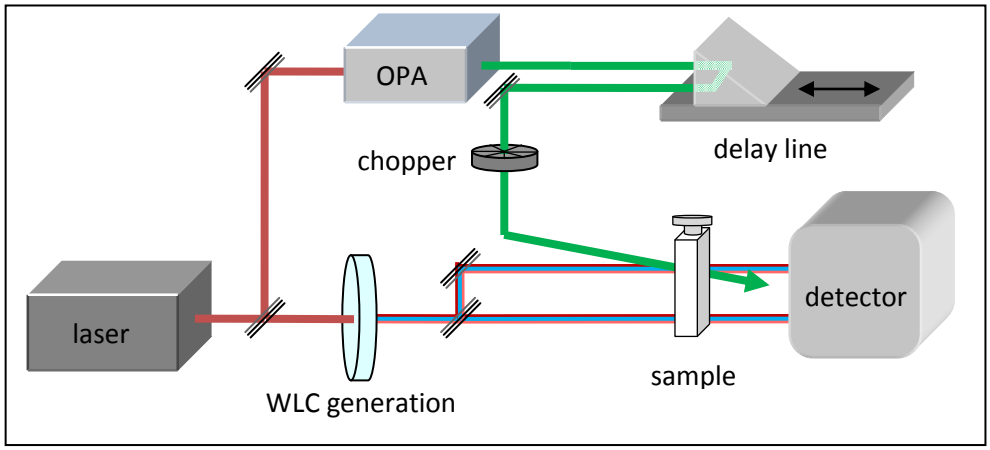

Fig 5. *General scheme of typical pump-probe experimental setup. See text for more details.*

region, a white-light continuum[57] is generated by means of self-phase modulation in a variety of materials and used as a broad-band probe beam. Alternatively, other conversion techniques such as harmonic generation or parametric amplification can be also used for varying the probe wavelengths if a single-wavelength probing regime is used. To resolve the measurement in time, a computer-controlled device called delay line is placed either in the pump or probe beam. By systematically increasing optical path in one of the beams, a certain

delay between pump and probe is introduced. Time delay Δt between the pump and probe pulses can be calculated using simple equation

$$\Delta t = \frac{\Delta l}{c_m} = \frac{n(l_{pr} - l_{pu})}{c}$$

where l_{pu} and l_{pr} are the optical paths of the pump and probe beams, Δl is their difference, c_m is speed of light in medium with refractive index n and c the speed of light in vacuum. It is noteworthy that to introduce delay Δt = 1 ps the calculated Δl is ~0.3 mm, thus when using a corner reflector that doubles the optical path, the desired displacement of the corner reflector for 1 ps delay (between pump and probe pulses) is approximately 150 μm.

Prior to its arrival to the sample, the probe beam is divided into probe and reference beams. The probe beam is then focused to the sample to spatially overlap with the pump beam, while the reference beam may or may not, depending on the type of experiment, pass the sample. After passing through the sample, both probe and reference beams are lead either to a photodiode (in the single-wavelength regime) or, when using the white-light probe, to a spectrograph where they are dispersed and detected by diode arrays to generate transient transmission spectrum of the sample. The transient absorption (TA) signal is then calculated using the following expression

$$TA = log\frac{I_{ref}}{I_{pp}} - log\frac{I_{ref}}{I_{np}}$$

where I_{pp} and I_{np} are the probe intensities with (pp) and without (np) excitation of the sample, and I_{ref} is the intensity of reference. Transient absorption signal measured as a function of the delay time between pump and probe beams monitors dynamics of transient species generated by excitation of the sample.

Many other examples of different pump-probe techniques can be found. Nowadays, even x-ray[58] or electron pulses may be used as the probe.[59] The only critical requirement is that the pulses in the pump beam are well synchronized to those in the probe beam and can be delayed in a controlled way.

3.2.2. Experimental setup

Majority of the time-resolved experiments described in this thesis were carried out with experimental setup shown in **Fig 6**. Femtosecond laser system Integra-i (Quantronix) was used as the source of short pulses. It is an integrated and

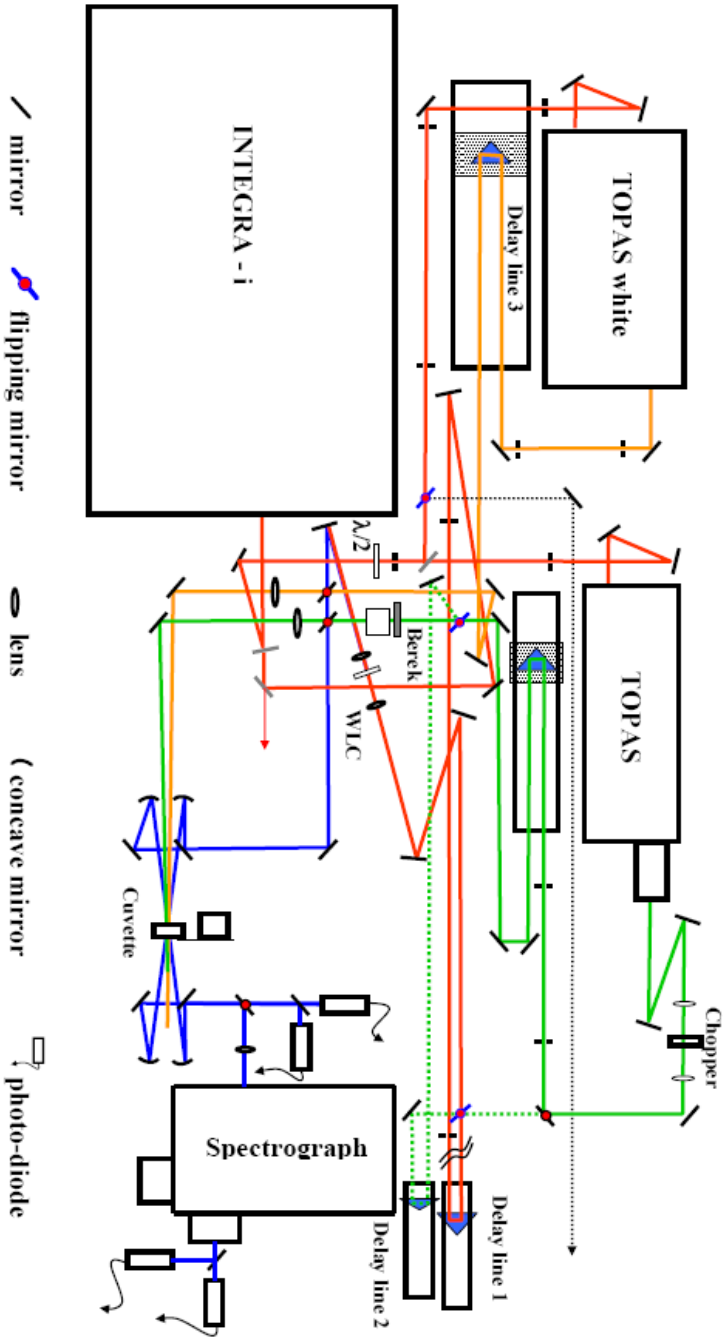

Fig 6. *Scheme of the experimental setup used for majority of the experiments.*

temperature-controlled unit based on primary Er-fiber oscillator generating low-intensity 80 fs pulses centered at 790 nm with 45 MHz repetition rate. These pulses are further amplified by a two stage (regenerative and multipass) Ti:sapphire amplifiers pumped by Nd:YLF nanosecond laser operating at frequency of 1 KHz. The amplifier system employs a chirped pulse amplification technique and can be divided into three major sections: the pulse stretcher, amplification of the stretched pulses in the regenerative and multipass amplifiers, and the pulse compressor. The Integra-i laser system provides pulses of ~130 fs duration centered at 790 nm with pulse energy of 2.1 mJ with 1 kHz repetition rate. The output beam was directed to a beamsplitter, which divides the primary beam into two branches. Reflected part was used for excitation ***(a)*** while transmitted part for probing ***(b)***.

(a) The laser beam for excitation was directed to a $\lambda/2$ plate to obtain polarization necessary for pumping the optical parametrical amplifier (TOPAS, Light Conversion) that enabled tuning of the excitation wavelength in a broad range spanning from UV to the near IR region (240 nm – 1150 nm) by sum-frequency generation and/or second and fourth harmonic generation, while keeping the excitation pulses reasonably short (<130 fs). After TOPAS, chopper was placed in the vicinity of focal point of a telescope, created by two convex lenses. This arrangement is important to avoid possible cutting-off the excitation pulses by the chopper blades. Slight off-focus position of chopper was chosen in order to avoid thermal degeneration of chopper blades since the flux densities *(number of photons passing through a unit of area per time unit – photons per pulse per cm^2)* may be sometimes too high in the focal plane. After passing the chopper, the excitation beam was reflected by dielectric mirrors, while the transmitted 790 nm leakage of the fundamental beam was dumped by laser attenuators. This helped to prevent photodamage of the optical elements and the sample itself without necessity of incorporating any additional filters, which may be a source of additional noise. The excitation beam was then directed to a flipping mirror, which, when flipped-in, reflected the beam to a corner retro-reflector mounted on non-motorized delay-line (for rough alignments), and further to the Berek compensator, used for setting the mutual polarization between pump and probe beams. When the flipping mirror was in out-of-beam position, the excitation beam was re-directed to the Berek compensator via delay line 2 (see **Fig 6** for more details). After the polarization adjustment, the excitation beam was focused by a long-focus (500-1000 mm), high-precision fused silica lens to the sample.

While the first described configuration utilizing delay line 1 was used for recording the pump-probe signals in the WLC-probe regime with diode array detection, the passage of the pump beam through the delay line 2 was chosen

when the single-wavelength kinetics were measured. Thus, it is obvious that it does not matter whereas the delay line is in the pump or in probe. The only important requirement is the respective delay between pump and probe pulses arriving at the sample.

(b) The part of the output beam that is transmitted through the first beam-splitter was used to generate white light continuum (WLC) for probing. Prior to WLC generation, the beams were sent around the optical table to compensate the long optical path of pump inside the TOPAS (~2.7 m) and then directed to the corner reflector of the delay line 1. The beam was then pointed to a short-focus convex lens which focused the beam to a 2 mm sapphire plate to generate WLC. The incident energies were kept low enough to generate a single-filament white light continuum. After collimation of the continuum by a 30 mm lens, generated WLC was directed to the sample area, usually passing through a hot mirror which strictly reflected wavelengths above ~780 nm, thus effectively eliminating the leakage of the fundamental beam (790 nm), which could be harmful for the sample or detector. The WLC was split into two parts by a 50/50 broadband beam-splitter to create probe (reflected part) and reference (transmitted part) beams that were both focused to the sample by a 300 mm spherical mirror in order to avoid dispersion of the WLC that occurs when passing through refractive optics. The probe beam was aligned to cross the path of the pump beam in the sample volume, while the reference beam was passing through the non-excited part of the sample (**Fig. 7**). After the sample, WLC probe and reference were collimated by another set of concave mirrors and directed to the entrance slit of a spectrograph, being dispersed using optical grating and finally detected by a pair of 1024-element diode-arrays. Transient absorption spectra were then calculated for each delay time using the equation described in section **3.2.1.**

To allow probing deeper in the blue part of spectrum, WLC was generated using a CaF_2 plate instead of sapphire. It is well-known that using CaF_2 shifts the generated WLC towards shorter wavelengths, allowing coverage of the UV region down to 350 nm. Since the CaF_2 crystal is, contrary to sapphire, easily damaged by incident photons, it was mounted in a home-built device providing a translation motion in the axis perpendicular to the incident beam. In such a case the polarization of WLC is similar to that of the incident laser light and the thermal degeneration of the crystal is prevented.

To conclude this section, it is important to stress that to prevent a thermal and photochemical degradation, the sample was placed in a rotating cuvette, operated by electric motor with a rubber gearing. The cuvette was of a sandwich-type, consisting of circular front and back windows, separated by a Teflon spacer. The front window of the cuvette was made of 1 mm thick high-precision fused

silica while the spacer thickness was dependent on particular experiment; typically 1 mm. Detail of the cuvette is shown in **Fig 7**. All measurements were carried out at room temperature.

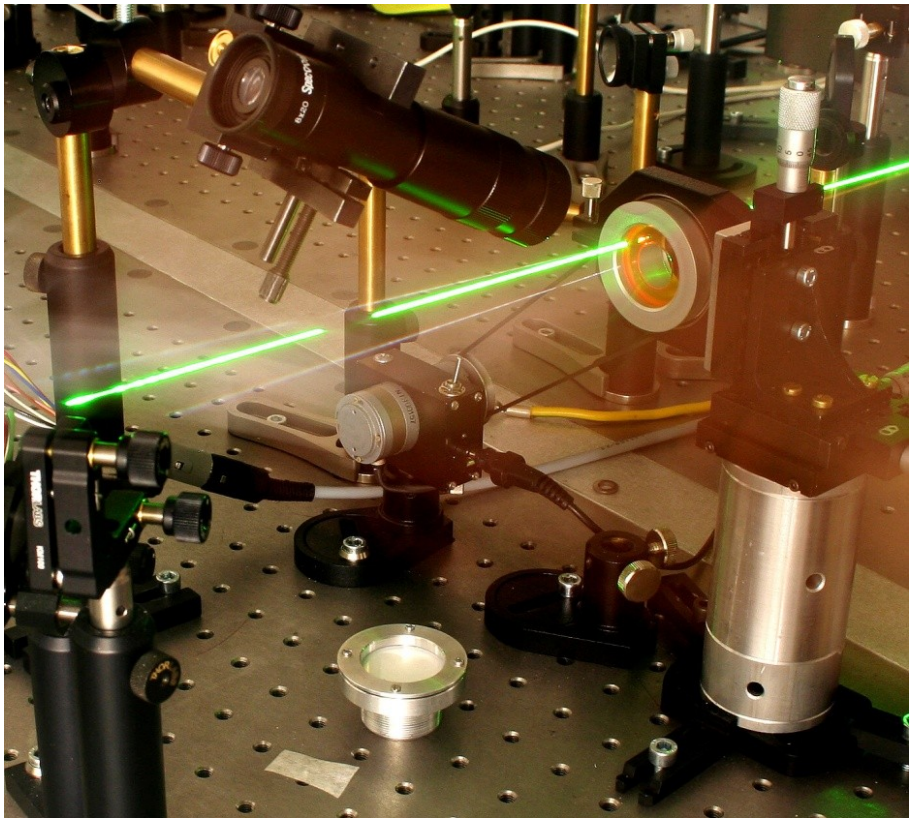

Fig 7. *Rotating cuvette with sample. The intense green beam is the pump while weaker white beams are WLC probe (crossing with pump in the sample) and reference.*

3.2.3. Pulse shaping (optimal control)

Various techniques for shaping, modulating or encoding of pulses are utilized for a variety of applications in communications, remote sensing or signal processing, and these techniques have already shown to be advantageous as an experimental tool providing valuable control over ultrafast laser pulses for ultrafast spectroscopy, nonlinear fiber optics, and high-field physics.[60] The

generation of properly shaped pulses is crucial for all of these applications. A number of approaches have been developed for this reason, yet the most used technique in the field of ultrafast optics is a Fourier synthesis to generate specially shaped ultrashort pulses. In the 1970's, Desbois *et al.*[61] and Agostinelli *et al.*[62] demonstrated a technique for optical pulse shaping based on spatial filtering of optical frequency components that were physically dispersed by diffraction gratings.

In the past decade the computer-controlled adaptive shaping of laser pulses become a powerful tool. The experimental output signals are used as feedback in an iterative learning loop which refines the applied laser field to fulfill the conditions given by user. Pulse shaping techniques are extremely useful for ultrafast spectroscopy while the properly shaped laser pulses allow manipulation with the populations of the excited states.

The most used pulse shapers are designed by a pulse stretcher in a 4*f* configuration in the way that the stretcher actually neither stretches nor compresses the pulses. A diffraction grating spreads the incident light and spatially separate different frequency (wavelength) components. Each frequency component is then focused at a particular spot in the focal plane. The second grating and a lens (or spherical mirror) are then used to recombine the different frequency components to resemble the shaped pulse (**Fig. 8**).

Pulse-shaping experiments described in this thesis were carried out at the Department of Chemical Physics in Lund, Sweden (**Paper V**). The pulse shaping of the pump pulses was provided by a 4f zero-dispersion grating compressor and liquid-crystal array placed in the Fourier plane of the compressor focus. The liquid crystals in the array were oriented along applied DC electric field introducing a voltage-dependent phase delay to the excitation pulses.[63] This particular pulse-shaping arrangement leads to altering the phases of individual colors of excitation laser pulses without changing their spectral composition (see upper part of **Fig 8** for more details).

The feedback loop controlled optimization was interactively searching for the best pump pulse shape in order to fulfill the experimental requirements in the most effective way, i.e. seeking for such a liquid-crystal mask that would shape the pump pulse in a way to, for instance, maximize population of a certain excited state (**Fig 8** - bottom). For characterization of temporal profiles of the optimized pulses a cross-correlation between the transform-limited (non-optimized) probe and optimized pump pulses technique was measured. For this reason, the BBO crystal (30-μm) was placed at the (mirror) sample position and the signal produced by sum-frequency generation between shaped pump and non-shaped

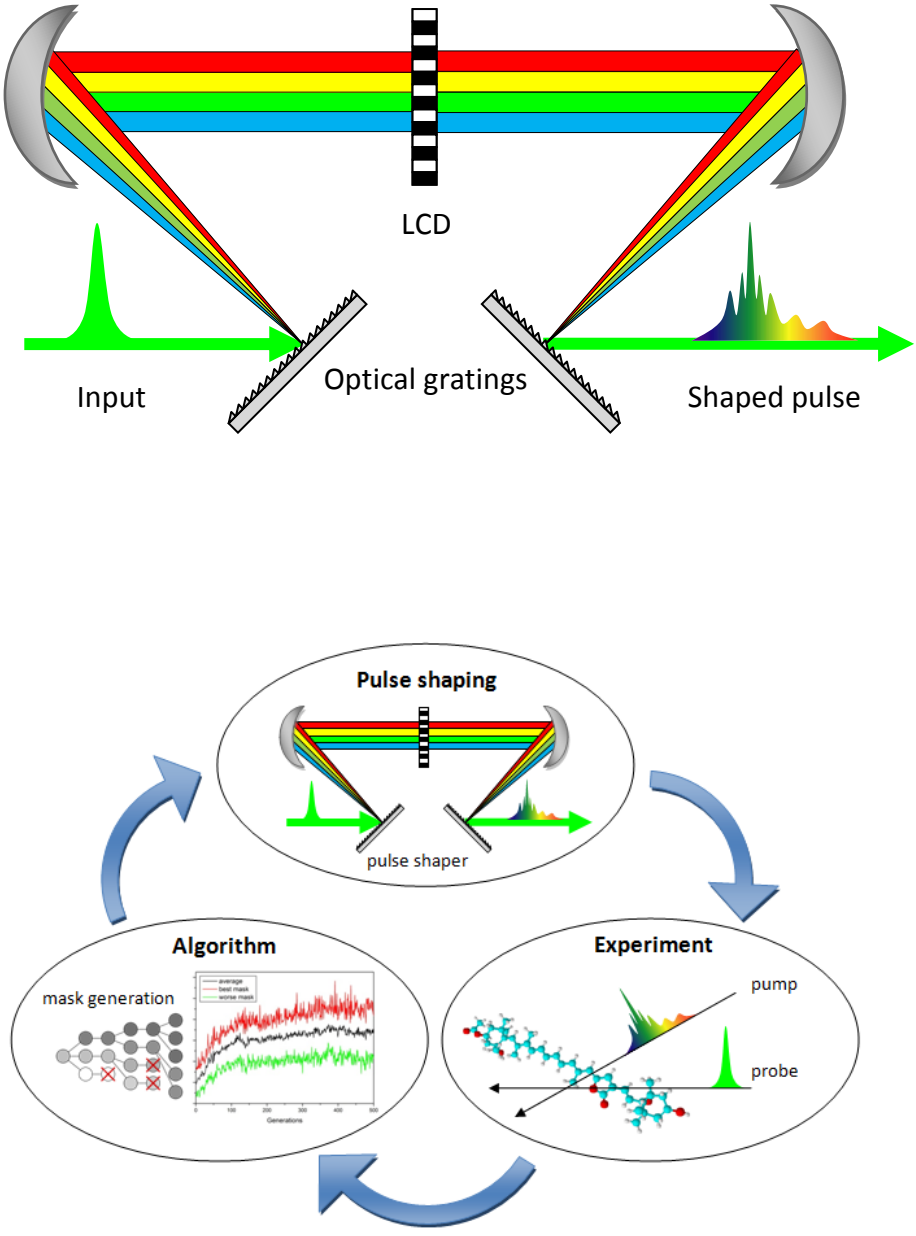

Fig 8. *Principle of the pulse shaper (top) and scheme of the control learning loop (bottom). See text for more details.*

probe pulses were detected by single photo-diode. Under these conditions it is possible to correlate directly the temporal profiles of the cross-correlation traces to the changes in the pump pulse profiles[64] resulting from the optimization process.

3.3. Data processing

All data collected by the diode-array detection system were processed by the data-fitting software DAFit (Pascher Instruments). This software is developed to meet the needs of femtosecond time-resolved pump-probe measurements with diode-array detection system. The raw data were loaded into the software in a matrix form containing the whole spectro-temporal datasets (wavelengths in columns, times in rows), and the software performed several important operations: subtracting the background, chirp correction and deconvolution of the instrument response function. It is worth noting that because the chirp phenomenon spoils all the multi-spectral measurements, the chirp-correction procedure is extremely important for data analysis. The reason for having the chirped (ultrashort) laser pulses lays in the fact that if the femtosecond polychromatic laser pulse passes through a medium with a certain refractive index, its spectral components are unequally delayed due to dispersion. Because the blue part of the pulse spectrum is more delayed than the red part, the resulting pulse is stretched and the time of interaction with the sample depends on the particular wavelength of the incident pulse. Since this delay between red and blue part of laser pulse is in the time frame of investigated dynamics, it is crucial to remove all chirp effects before proceeding with the data analysis.

After performing the above mentioned corrections the fitting program allows to use up to five exponential components, whose time constants are entered as initial guesses. The spectro-temporal datasets are then fitted to a sum of exponentials

$$\sum_n A_n\,(\lambda)\,exp(-k_n t)$$

where *n* varies from 1 and 5. This procedure, usually called global fitting,[65] allows for better determination of the time constants of the excited state processes and for assignment of spectral profiles of the intermediate excited-state species. When modeling the excited state dynamics we assume, for simplicity, that the excited system is evolving according to an irreversible sequential model

$$A \overset{k_1}{\rightarrow} B, \qquad B \overset{k_2}{\rightarrow} C, \qquad C \overset{k_3}{\rightarrow} D \ldots$$

where A, B, C and D represent excited state species and k_1, k_2 and k_3 are the rate constants characterizing the monoexponential decay time of population of a particular species. The separated spectral profiles of the individual excited-state species are usually known as evolution-associated difference spectra (EADS). Even though for complicated systems that may include reversible and/or branching processes the EADS do not correspond to spectra of true excited states, they are still able to provide valuable information about excited-state dynamics of the studied system.[65]

4. Summary of papers

In **Paper I** the dependence of excited-state properties of carbonyl carotenoids on the molecular structure was examined. We compared excited-state dynamics of four carotenoids with nearly identical structure, differing only by number and/or position of conjugated carbonyl groups. Besides observed effect of carotenoid S_1 lifetime shortening upon increasing number of conjugated carbonyl groups we have focused on appearance of the S* bands in transient signals. On the basis of measured data we sustain the hypothesis that the S* state is preferentially populated for molecules with specific ground state conformation, demonstrated here in **Fig 9** (not included in the paper). Two types of ground state conformers are likely present in carotenoid solution ("type A" and "type B" in **Fig 9**). After excitation of molecule A, the S_1 state is populated and (standard) S_1-S_n band observed. After excitation molecule B undergoes an internal conversion followed by a molecular twist into slightly different molecular conformation, populating the S* state and the S*-S_N band (S* band) appears in transient data. The lower part of **Fig 9** shows normalized transient spectra (rhodoxanthin in benzene) taken at early times after excitation, where both A and B type molecules are excited. Spectrum is rather featureless in the bleaching region and has two ESA bands, originating from S_1-S_n and S*-S_N transitions, respectively (black line). After some time (τ_A) the S_1 state relaxes back to the ground state, but because $\tau_A < \tau_B$, the S* signal is still observed (red line). The fact that the signal originates from a specific set of molecules (here, only from molecules B) is demonstrated by the presence of pronounced vibronic structure in the bleaching region.

Excited state properties of water-soluble carotenoids were investigated in **Paper II**. The hydrophilicity of these carotenoids allowed us to extend studies of polarity-dependent behavior to solvent with extreme polarity, water. We have shown that for a solvent with very high polarity the lifetime shortening can be induced even in carbonyl carotenoid with $N > 10$, an effect that has not been observed before. Interestingly, the observed polarity-induced S_1 lifetime shortening is not accompanied with appearance of ICT bands in the transient absorption spectrum, also contradicting the earlier expectations. As the conjugation length and solvent polarity seems to be insufficient to explain appearance and character of the ICT state, we suggested the structures of carotenoid molecules to be responsible for these phenomena. Based on previous studies we assumed the S_{ni} state to be of $1A_g^+$ symmetry (the "cis-peak"). Thus,

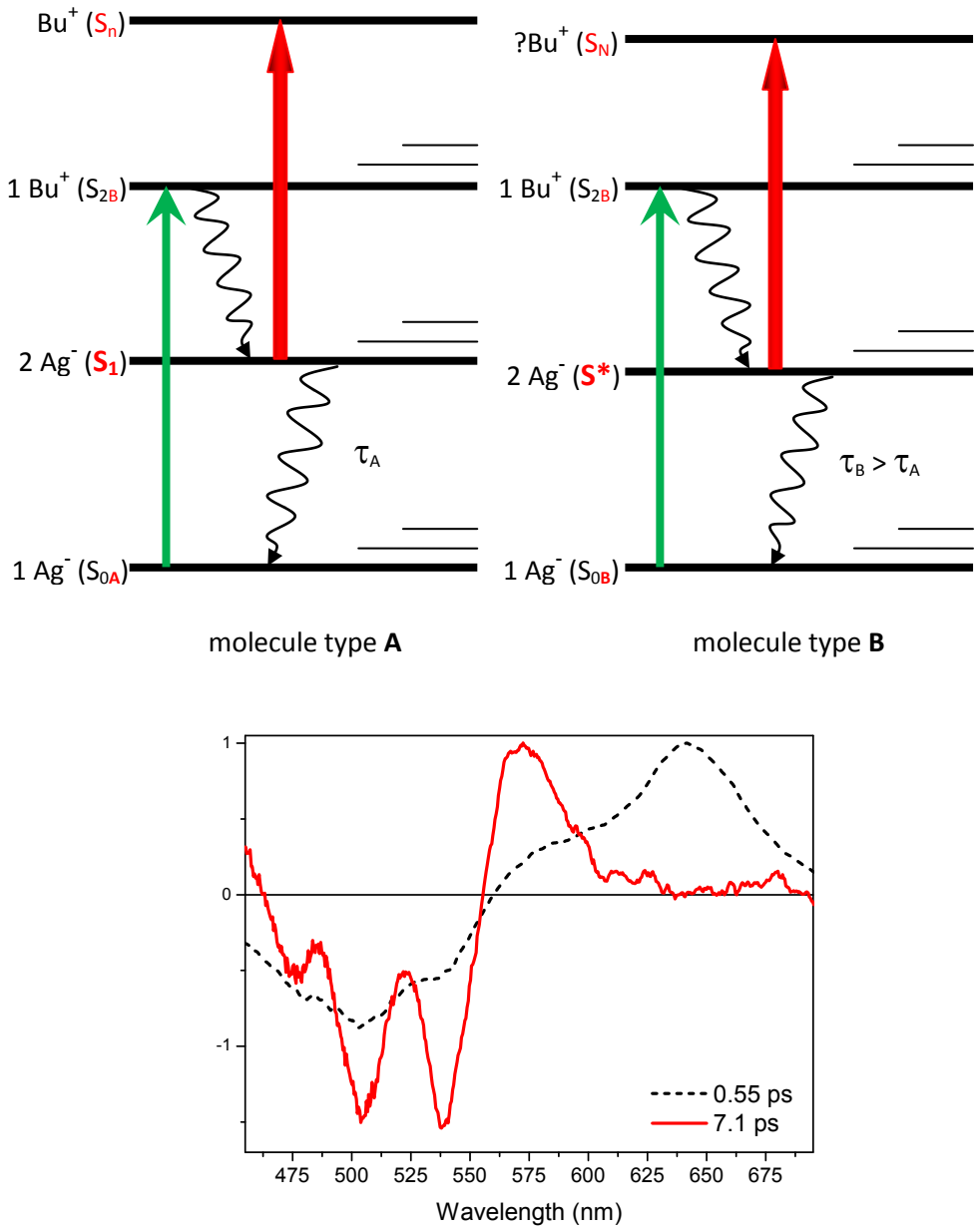

Fig 9. *"Inhomogeneity model" of the S* state proposed in **Paper I** (top) and transient absorption spectra of rhodoxanthin in benzene at 0.55 ps, where "type **A** and **B**" are populated and spectra at 7.1 ps, where population of **B** dominates. Spectra are normalized to their maxima.*

transition from the ICT ($2\ Ag^-$) to S_{ni} ($1A_g^+$), which responsible for the ICT band in transient spectra, is allowed only upon symmetry breaking conditions (see **Fig. 10**)

We have concluded that asymmetrical position of the carbonyl group(s) is a key requirement for the occurrence of the ICT band in the transient absorption spectra, and that the magnitude of the ICT band is a measure of the asymmetry in electron distribution in the ICT state.

Paper III focuses on influence of the carotenoid end rings. We investigated the effect of ϕ-rings on excited state properties of carotenoid. It was shown that when b-ring is replaced by ϕ-ring, which contain two extra conjugated C=C bonds, the effective conjugation length decreases. This unexpected observation is explained by isolation of the π-electrons within the ϕ-ring by creating a local conjugation that is detached from the main conjugated backbone, most likely due to a twist of the terminal ring out of the molecular plane. Consequently, when comparing a carotenoid having β-rings at the ends with its analogue possessing ϕ-rings, the S_1 lifetime is systematically longer for the carotenoid without ϕ-rings, proclaiming the shorter effective conjugation length.

In **Paper IV** we have applied the pulse-shaping technique in attempt to control the excited-state properties of carbonyl carotenoid peridinin. Although we achieved control to decrease population of the S_1 or ICT states by shaping the excitation pulse, it was not possible to change the population ratio or to increase population of the S_1 or ICT states. The achieved decreasing of the population we explain as a dumping effect of the shaped pump pulse, which first part excite the molecule, while the latter part dumps the population back to the ground state. Fourier transform analysis of the optimized pulses allowed to identify low-energy vibrational modes involved in deactivation of peridinin excited states.

Paper V focuses on a novel antenna complex xanthorhodopsin, which is the simplest photosynthetic antenna known so far. To investigate the influence of carotenoid to protein binding, we treated xanthorhodopsin with $NaBH_4$, which shifts the energy of the acceptor state to higher energies and consequently prevents the energy transfer without distortion of the binding site. No S_1-mediated energy transfer channel was found as evidenced by identical S_1 lifetime in $NaBH_4$ treated and native xanthorhodopsin. By comparing $NaBH_4$ treated and native xanthorhodopsin we have identified ultrafast energy transfer via salinixanthin S_2 state operating with 40% efficiency.

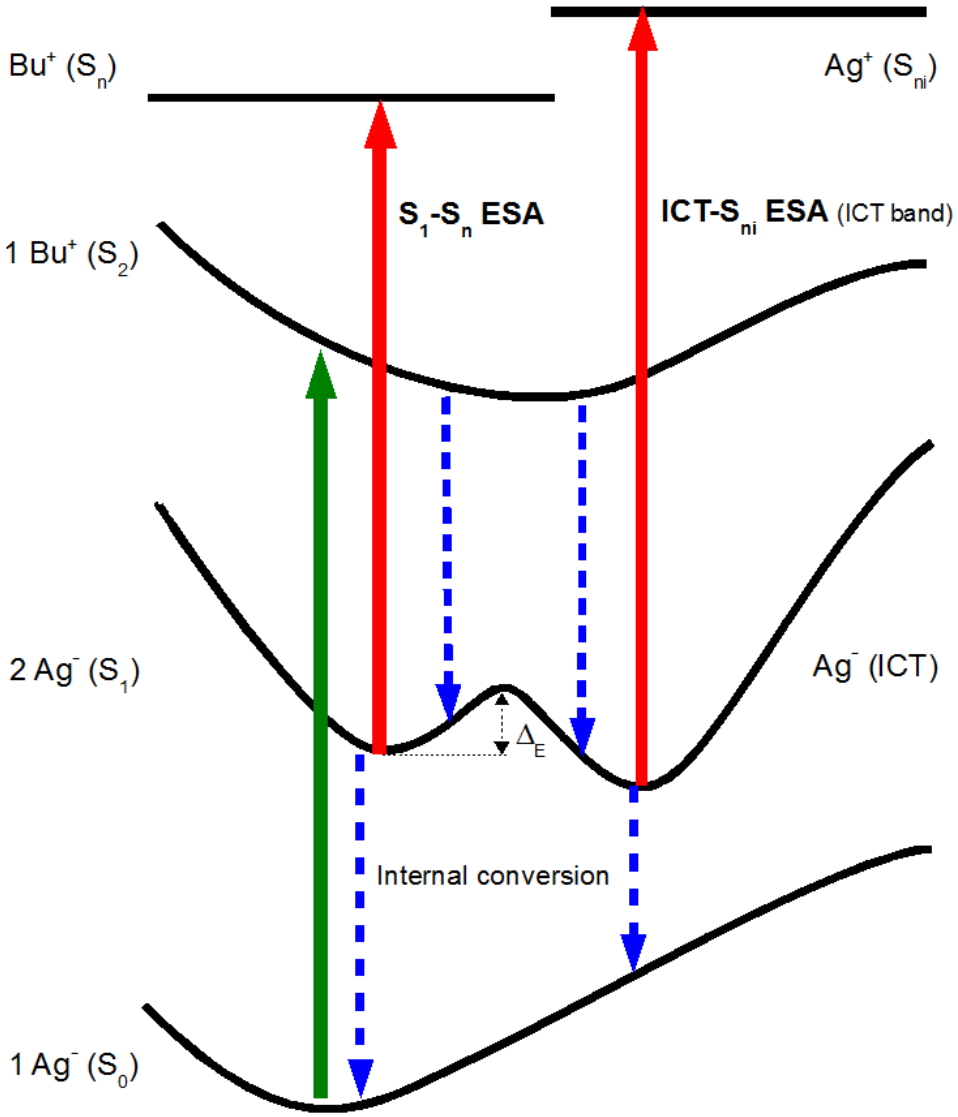

Fig 10. *Proposed model of excited states of a carbonyl carotenoid. After excitation (full green arrow) the S_1 and ICT states are populated by branching process from the S_2 state (blue dashed arrows). The S_1 and ICT states are separated by energetical barrier, which height is driven by solvent polarity. The ICT-S_{ni} transition (A_g^--A_g^+) is allowed only upon symmetry breaking conditions. See text for details.*

In **Paper VI** we compared excited-state properties of the carbonyl carotenoid 3'-hydroxyechinenone bound in red carotenoid protein (RCP) and orange carotenoid protein (OCP). It is shown that missing C-terminal domain in RCP, which provides a binding cleft for the carbonyl group of hydroxyechinenone in OCP, induces structural change of hydroxyechinenone. While in OCP the terminal ring containing conjugated carbonyl group is planarized due to binding to the C-terminal domain, in RCP the protein lock is released, returning the excited-state properties of hydroxyechinenone to those observed in solution.

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I. **Effect of carotenoid structure on excited-state dynamics of carbonyl carotenoids,** P. Chábera, M. Fuciman, P. Hříbek and T. Polívka, *Phys. Chem. Chem. Phys*., **2009**, 11, 8795-8803; **80 %**

Abstract (EN)

Effects of introducing a carbonyl group and its position in the conjugated system of carotenoids were studied by means of femtosecond time-resolved spectroscopy. We have compared four naturally occurring carotenoids with comparable structures, β-carotene, echinenone, canthaxanthin and rhodoxanthin, which differ in the number and position of conjugated carbonyl group(s). The S_1 lifetime is systematically shorter upon increasing the number of the conjugated C=O groups, yielding 9.3 ps (for β–carotene, no C=O group), 6.2 ps (echinenone, one C=O group), 4.5 ps (canthaxanthin, two C=O groups), and 1.1 ps (rhodoxanthin, two C=O groups in s-trans configuration). Except for slight polarity-induced broadening of absorption and transient absorption spectra, no other polarity effects, such as shortening of the S_1 lifetimes or transient features attributable to intramolecular charge transfer (ICT) state bands, were observed. The absence of these polarity-induced features is explained as due to the long conjugated chain (no lifetime shortening), and the symmetrical position of the carbonyl groups (no ICT bands). On the other hand, all carotenoids exhibit the characteristic spectral band attributed to the S* state, and for the two longest carotenoids, canthaxanthin and rhodoxanthin, decay of the S* state is markedly longer than that of the S_1 state. Moreover, it is shown that the S* state is preferentially populated for a specific subset of ground state conformations, underlining the importance of carotenoid conformation in S* state formation.

Abstrakt (CZ)

Efekty karbonylové skupiny a její pozice v konjugovaném systému karotenoidů byly studovány pomocí femtosekundové, časově rozlišené spektroskopie. Srovnali jsme čtyři přirozeně se vyskytující karotenoidy se srovnatelnou strukturou, β-caroten, echinenon, canthaxanthin a rhodoxanthin, kreré se liší počtem a pozicí karbonylových skupin. Doba života excitovaného S_1 stavu je systematicky kratší se zvyšujícím se počtem konjugovaných C=O skupin, dosahující 9.3 ps (β−caroten, bez C=O skupin), 6.2 ps (echinenon, jedna C=O skupina), 4.5 ps (canthaxanthin, dvě C=O skupiny) a 1.1 ps (rhodoxanthin, dvě C=O skupiny v s-trans konfiguraci). Kromě malého, polaritou vyvolaného, rozšíření transientního absorpčního spektra, žádné jiné změny v závislosti na polaritě, jako například zkracování doby života S_1 stavu či spektrální pásy stavu s přenosem náboje (intramolecular charge tranfer, ICT stav) nebyly pozorovány. Nepřítomnost těchto efektů je vysvětlena dlouhým konjugovaným řetězcem karotenoidu (nedochází ke zkracování doby života S_1 stavu) a symetrickou pozicí karbonylových skupin (neobjeví se ICT pásy). Všechny karotenoidy ale vykazují spektrální pás příslušející S* stavu a pro dva nejdelší karotenoidy, canthaxanthin a rhodoxanthin, je doba života S* stavu výrazně delší než doba života S_1 stavu. Navíc je ukázáno, že S* stav je přednostně populován ve skupině molekul se specifickou konformací v základním stavu, což podtrhuje důležitost molekulární konformace karotenoidu při formaci S* stavu.

II. **Ultrafast dynamics of hydrophilic carbonyl carotenoids - relation between structure and excited state properties in polar solvents**, P. Chábera, M. Fuciman, K. R. Naqvi and T. Polívka, *Chem Phys.,* **2010**, **doi**: 10.1016/ j.chemphys.2010.01.007; **80 %**

Abstract (EN)

We present a study of excited-state dynamics of water-soluble carbonyl carotenoids, crocin, norbixin, and astalysine in solvents with different polarity. While no polarity effects were observed in 2-propanol and methanol, polarity-induced lifetime shortening has been detected in water. For crocin and astalysine the S_1 lifetime decreases from 135 ps to 61 ps (crocin), and from 4 ps to 2.2 ps (astalysine) when going from methanol to water. The S_1 lifetime of norbixin is within the 15–18 ps range in all solvents, an effect attributed to its carboxylic group, which isolates the carbonyl group from the rest of conjugation. No spectral bands attributable to the intramolecular charge transfer (ICT) state have been found in any transient absorption spectra. The ICT–S_n transition is made forbidden, we suggest, by the symmetric location of the conjugated carbonyl groups. In astalysine, we have found a clear signature of the S* state with a lifetime of 7 ps (methanol) and 6.1 ps (water).

Abstrakt (CZ)

Tato práce popisuje studii excitovaných stavů hydrofilních karbonylových karotenoidů (crocin, norbixin a astalysin) v rozpouštědlech s různou polaritou. Zatímco žádné polaritou vyvolané změny nebyly detekovány v 2-propanolu a metanolu, ve vodě bylo pozorováno zkracování doby života S_1 stavu vyvolané polaritou rozpouštědla. U crocinu a astalysinu se doba života S_1 zmenšila ze 135 ps na 61 ps (crocin), ze 4 ps na 2.2 ps (astalysin) při změně rozpouštědla z metanolu na vodu. Doba života norbixinu zůstává v rozmezí 15-18 ps ve všech rozpouštědlech, což je

vysvětleno vlivem karboxylové skupiny, která izoluje karbonylovou skupinu od zbytku konjugovaného řetězce. Žádné pásy příslušející ICT (intramolecular charge transfer) stavu nebyly pozorovány v žádných transientních absorpčních spektrech. Předpokládáme, že ICT–S_n přechod je zakázán díky symetrickému umístění konjugovaných karbonylových skupin. V astalysinu jsme našli jasné známky S* stavu s dobou života 7 ps v metanolu a 6.1 ps ve vodě.

III. **Excited state properties of aryl carotenoids,** M. Fuciman. P. Chábera, A. Župčanová, P. Hříbek, J. B. Arellano, F. Vácha, J. Pšenčík and T. Polívka, *Phys. Chem. Chem. Phys*., **2010**, 12, 3112-3120; **30 %**

Abstract (EN)

Excited-state properties of aryl carotenoids, important components of light harvesting antennae of green sulphur bacteria, have been studied by femtosecond transient absorption spectroscopy. To explore effects of the conjugated aryl group, we have studied a series of aryl carotenoids with conjugated ϕ-ring, chlorobactene, β-isorenieratene and isorenieratene, and compared them with their non-aryl counterparts γ-carotene and β-carotene, which contain β-ring. Changing β-ring to ϕ-ring did not reveal any changes in absorption spectra, indicating negligible effect of the ϕ-ring on the effective conjugation length. This observation is further supported by the carotenoid S_1 lifetimes. In *n*-hexane, the S_1 lifetime of chlorobactene having one ϕ-ring is 6.7 ps, while the S_1 lifetime of the β-ring analog, γ-carotene is 5.4 ps. The same effect is observed for the series β-carotene (2 β-rings), β-isorenieratene (1 β- and 1 ϕ-ring) and isorenieratene (2 ϕ-rings) whose S_1 lifetimes in *n*-hexane are 8.2, 10.3 and 12.7 ps, respectively. The systematically longer lifetimes of aryl carotenoids show that the additional conjugated C=C bonds at the ϕ-ring do not contribute to the conjugation length. The S_1 lifetimes of aryl carotenoids were slightly shortened in benzene, indicating π-π stacking interaction between the ϕ-ring and benzene.

Abstrakt (CZ)

Vlastnosti excitovaných stavů arylových karotenoidů, které se běžně vyskytují ve světlosběrných anténách zelených sirných bakterií, byly studovány pomocí femtosekundové, transientní

absorpční spektroskopie. Pro zjištění vlivu konjugované arylové skupiny na vlastnosti excitovaných stavů jsme použili sérii arylových karotenoidů s konjugovaným ϕ-kruhem: chlorobacten, β-isorenieraten a isorenieraten, a tyto následně porovnali s jejich nearylovými protějšky γ-carotenem a β-carotenem, které obsahují β-kruh. Změna β-kruhu na ϕ-kruh nezpůsobila žádné změny v absorpčních spektrech, což ukazuje na zanedbatelný vliv ϕ-kruhu na efektivní konjugovanou délku karotenoidu. Tato pozorování jsou ještě dále potvrzena měřením doby života S_1 stavu. V *n*-hexanu je doba života S_1 stavu chlorobactenu (jeden ϕ-kruh) 6.7 ps, zatímco doba života S_1 stavu γ-carotenu s β-kruhuem je 5.4 ps. Tentýž efekt byl pozorován u série β-caroten (2 β-kruhy), β-isorenieraten (1 β- a 1 ϕ-kruh) a isorenieraten (2 ϕ-kruhy), jejichž doby života S_1 stavu v *n*-hexanu jsou 8.2, 10.3 a 12.7 ps. Systematicky delší doby života S_1 stavu u arylovýh karotenoidů ukazují, že dodatečné konjugované C=O skupiny na ϕ-kruhu nepřispívají ke konjugované délce karotenoidu. Kratší doby života stavu S_1 stavuu arylových karotenoidů mohou být vysvětleny π-π interakcí mezi ϕ-kruhy a benzenem.

IV. **Optimal Control of Peridinin Excited-State Dynamics,** B. Dietzek, P. Chábera, R. Hanf, S. Tschierlei, J. Popp, T. Pascher, A. Yartsev and T. Polívka, *Chem. Phys.*, **2010**, **doi**:10.1016/j.chemphys.2010.02.028; **40 %**

Abstract (EN)

Optimal control is applied to study the excited-state relaxation of the carbonyl carotenoid peridinin in solution. Phase-shaping of the excitation-pulses is employed to influence the photoinduced reaction dynamics of peridinin. The outcome of various control experiments using different experimentally imposed fitness parameters is discussed. Furthermore, the effects of pump-wavelength and different solvents on the control efficiency are presented. The data show that excited-state population within either the S_1 or the ICT state can be reduced significantly by applying optimal control, while the efficiency of control decreases upon excitation into the low-energy side of the absorption band. However, we are unable to alter the ratio of S_1 and ICT population or increase the population of either state compared to excitation with a transform-limited pulse. We compare the results to various control mechanisms and argue that characteristic low-wavenumber modes are relevant for the photochemistry of peridinin.

Abstrakt (CZ)

Metoda tvarování femtosekundových pulsů byla použita při studiu relaxace excitovaných stavů karbonylového karotenoidu peridininu. K ovlivnění světlem vyvolané reakční dynamiky peridininu bylo použito modulování fáze excitačních pulzů. V článku jsou diskutovány výsledky různých experimentů s různými experimentálními parametry. Rovněž jsou popsány efekty změny vlnové délky excitace a změny polarity rozpouštědla na výsledky experimentu. Data ukazují, že populace excitovaných stavů S_1 a ICT mohou být významně redukovány za použití techniky tvarování

pulsů. Úspěšnost experimentu (= optimalizace výstupních parametrů pomocí tvarování pulsů) se snižuje při excitaci delšími vlnovými délkami. V žádném z experimentů nebylo možné dosáhnout změny poměru populací S_1 a ICT stavů nebo zvýšit jejich populaci v porovnání s použitím nemodulovaného pulzu. Srovnáním výsledků sady experimentů lze dojít k závěru, že charakteristické nízkofrekvenční módy jsou důležité pro fotochemii peridininu.

V. **Femtosecond Carotenoid to Retinal Energy Transfer in Xanthorhodopsin**, T. Polívka, S. P. Balashov, P. Chábera, E. S. Imasheva, A. Yartsev, V. Sundström and J. K. Lanyi, *Biophys. J.,* **2009**, 96, 2268-2277; **20 %**

Abstract (EN)

of the extremely halophilic bacterium Salinibacter ruber represents a novel antenna system. It consists of a carbonyl carotenoid, salinixanthin, bound to a retinal protein that serves as a light-driven transmembrane proton pump similar to bacteriorhodopsin of archaea. Here we apply the femtosecond transient absorption technique to reveal the excited-state dynamics of salinixanthin both in solution and in xanthorhodopsin. The results not only disclose extremely fast energy transfer rates and pathways, they also reveal effects of the binding site on the excited-state properties of the carotenoid. We compared the excited-state dynamics of salinixanthin in xanthorhodopsin and in $NaBH_4$-treated xanthorhodopsin. The $NaBH_4$ treatment prevents energy transfer without perturbing the carotenoid binding site, and allows observation of changes in salinixanthin excited-state dynamics related to specific binding. The S_1 lifetimes of salinixanthin in untreated and $NaBH_4$-treated xanthorhodopsin were identical (3 ps), confirming the absence of the S_1-mediated energy transfer. The kinetics of salinixanthin S_2 decay probed in the near-infrared region demonstrated a change of the S_2 lifetime from 66 fs in untreated xanthorhodopsin to 110 fs in the $NaBH_4$-treated protein. This corresponds to a salinixanthin-retinal energy transfer time of 165 fs and an efficiency of 40%. In addition, binding of salinixanthin to xanthorhodopsin increases the population of the S* state that decays in 6 ps predominantly to the ground state, but a small fraction (<10%) of the S* state generates a triplet state.

Abstrakt (CZ)

Xanthorhodopsin z extrémně halofilní bakterie *Salinibacter ruber* je unikátní anténní systém, který se skládá z karbonylového karotenoidu salinixanthinu a pigmentu retinalu. Xanthorhodopsin funguje, podobně jako bakteriorhodopsin, jako světlem řízená transmembránová protonová pumpa. V této práci je použita techniku femtosekundové transientní absoprce k odhalení dynamiky excitovaných stavů salinixanthinu jak v roztoku tak v xanthorhodopsinu. Výsledky ukazují na extrémně rychlý přenos energie mezi salinixanthinem a retinalem, ale také odhalují vliv vazebného místa proteinu na dynamiku excitovaných stavů salinixanthinu. Dynamiku excitovaných stavů salinixanthinu v xanthorhodopsinu je srovnána s identickými procesy v xanthorhodopsinu vystaveném působení $NaBH_4$. Použití $NaBH_4$ znemožňuje přenos energie, ale zachovává neporušené vazebné místo salinixanthinu, čímž umožňuje pozorování změn v dynamice excitovaných stavů salinixanthinu v závislosti na vazbě karotenoidu na protein. Doba života S_1 stavu salinixanthinu je shodná v nativním xanthorhodospinu i v xanthorhodopsinu ovlivněném $NaBH_4$. , což potvrzuje hypotézu, že S_1 stav se neúčastní přenosu energie. Kinetiky dohasínání S_2 stavu salinixanthinu v infračervené oblasti odhalily změnu doby života S_2 stavu ze 66 fs v nativním xanthorhodopisnu na 110 fs v xanthorhodopsinu vystaveném působení $NaBH_4$. Tato změna odpovídá přenosu energie ze salinixanthinu na retinal s časovou konstantou 165 fs a účinností 40 %. Vazba salinixanthinu na xanthorhodopsin navíc zvyšuje populaci S* stavu, který dohasíná v 6 ps převážně do základního stavu, zatímco malá část (<10%) populace S* generuje tripletní stav.

VI. **Excited-state properties of the 16 kDa red carotenoid protein from *Arthrospira maxima***, P. Chábera, M. Durchan, C. A. Kerfeld and T. Polívka, submitted to *BBA – Bioenergetics*; **50 %**

Abstract (EN)

We have studied spectroscopic properties of the 16 kDa red carotenoid protein (RCP), which is closely related to the orange carotenoid protein (OCP) from cyanobacteria. Both proteins bind the same chromophore, the carotenoid 3'hydroxyechinenone (hECN), and the major difference between the two proteins is lack of the C-terminal domain in the RCP; this results in exposure of part of the carotenoid. The excited-state lifetime of hECN in the RCP is 5.5 ps, which is markedly longer than in OCP (3.3 ps) but close to 6 ps obtained for hECN in organic solvent. This confirms that the binding of hECN to the C-terminal domain in the OCP changes conformation of hECN, thereby altering its excited state properties. A hydrogen bond between the C-terminal domain and the carotenoid in the OCP is also absent in the RCP. This allows the conformation of hECN in the RCP to be similar to that in solution, which results in comparable excited-state properties of hECN in solution. The red-shift of the RCP absorption spectrum is most likely due to aggregation of RCP induced by hydrophobic nature of hECN that, when exposed to buffer, stimulates formation of assemblies minimizing contact of hECN with water. We suggest that the loss of the C-terminal domain renders the protein amphipathic, containing both hydrophobic (the exposed part of hECN) and hydrophilic (N-terminal domain) regions, may help the RCP to interact with lipid membranes; exposed hECN can penetrate into the hydrophobic environment of the lipid membrane, possibly to provide additional photoprotection.

Abstrakt (CZ)

Tato práce popisuje studii spektroskopických vlastností 16 kDa červeného karotenoidového proteinu (red carotenoid protein, RCP), který je blízce příbuzný podobnému proteinu OCP (orange carotenoid protein, OCP). Oba proteiny se běžně vyskytují v sinicích a váží stejný pigment, karotenoid 3'-hydroxyechinenon (hECN). Zásadní rozdíl mezi těmito dvěmi proteiny je, že RCP postrádá C-terminal doménu, což vede k vystavení části karotenoidu vlivům prostředí. Doba života S_1 stavu hECN v RCP je 5.5 ps, což je významně delší než v OCP (3.3 ps), ale blízko k 6 ps získaných pro hECN v organickém rozpouštědle. To potvrzuje, že vazba hECN na C-terminal doménu OCP změní konformaci hECN a tudíž i vlastnosti excitovaných stavů. Vodíková vazba mezi amionokyselinami v C-terminal doméně a karotenoidem v RCP chybí. Absence vodíkové vazby je základní příčinou shodné konformace hECN v RCP a v roztoku, což je příčinou podobných vlastostí excitovaných stavů hECN v RCP a v roztoku. Červený posun absorpčního spektra RCP je pravděpodobně způsoben agregací RCP, vyvolanou hydrofobní povahou hECN. Vystavení hECN pufru stimuluje vytvoření sekupení minimalizujících konktakt s vodou. Ztráta C-terminal domény způsobí, že RCP obsahuje hydrofobní (obnažená část hECN) i hydrofilní (N-terminal doména) oblasti, což může vést k lepší interakci s lipidovými membránami. Hydrofobní část RCP může pronikat do hydrofobního prostředí lipidních membrán, kde může například plnit fotoprotektivní funkci.