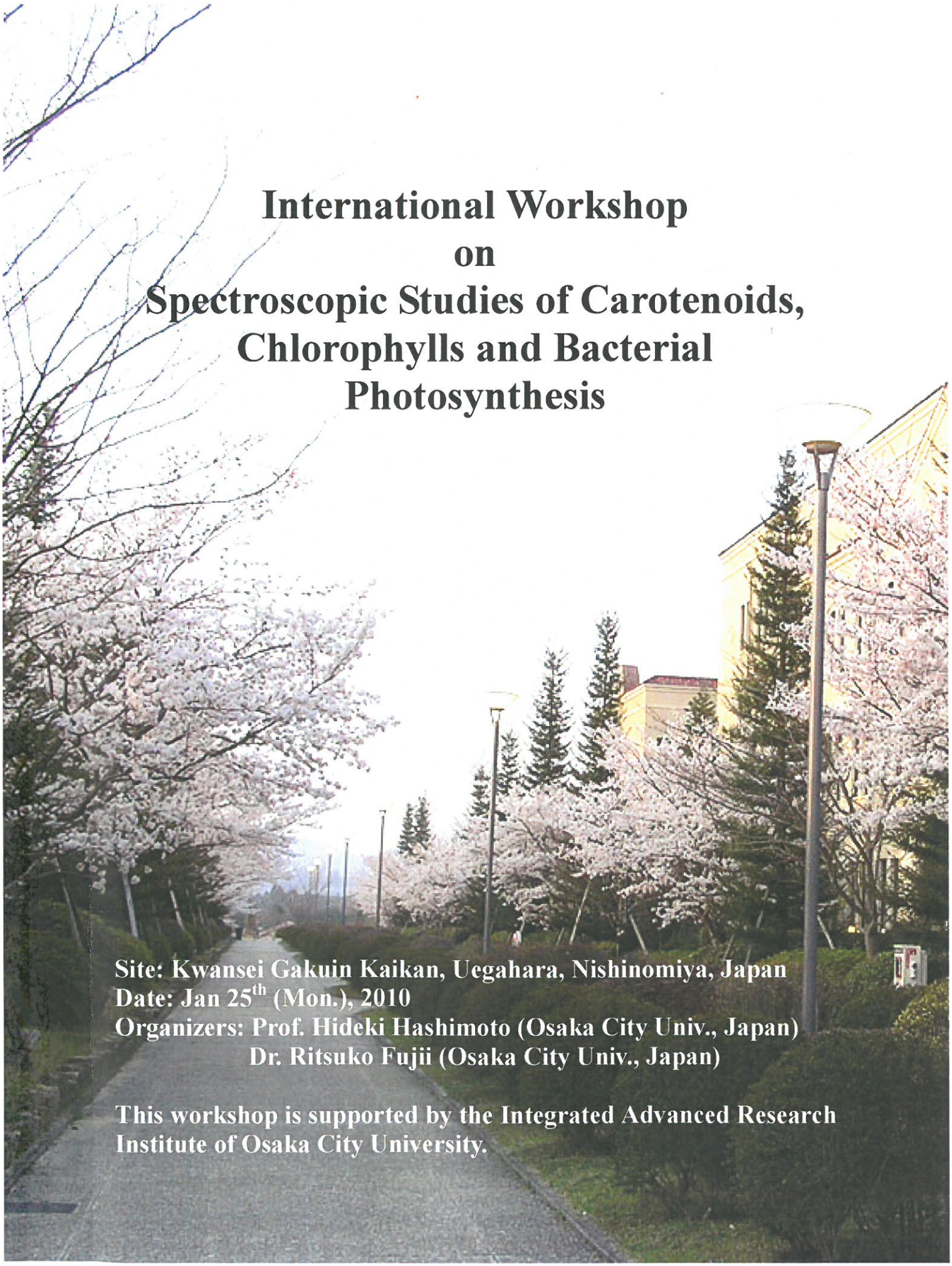
# International Workshop on Spectroscopic Studies of Carotenoids, Chlorophylls and Bacterial Photosynthesis



**Site: Kwansei Gakuin Kaikan, Uegahara, Nishinomiya, Japan**
**Date: Jan 25th (Mon.), 2010**
**Organizers: Prof. Hideki Hashimoto (Osaka City Univ., Japan)**
**Dr. Ritsuko Fujii (Osaka City Univ., Japan)**

**This workshop is supported by the Integrated Advanced Research Institute of Osaka City University.**

**Preface**

Photosynthesis is a crucial biological process as the primary resource on the earth. It converts light energy from the sun into chemical energy, and provides a food source for all higher lives. All fossil fuels have been produced by the photosynthetic processes, and oxygenic photosynthesis changed the atmosphere from anoxic to oxygen-rich 2.5 billion years ago. Therefore, the molecular mechanism of photosynthetic processes should be elucidated to bring forth a breakthrough to produce new and sustainable energy resources.

Primary processes of photosynthesis are performed by photosynthetic pigments, such as carotenoids and (bacterio)chlorophylls, bound to the pigment-protein complexes. Prof. Koyama dedicated his 30 years of research life to elucidate the molecular mechanism of the primary processes in bacterial photosynthesis using spectroscopic approaches. He is going to mandatory retire from Kwansei Gakuin University in the end of March 2010. In this opportunity, we invited Prof. Koyama and his past and recent collaborators, who are eminent in their field, to hold the international workshop on "Spectroscopic Studies of Carotenoids, Chlorophylls and Bacterial Photosynthesis". In this workshop, we discuss about recent research topics concerning with the photosynthetic pigments, following the footsteps of his entire research life.

Shortage of energy and water resources (= food production) and the global warming are the global crises that the human beings on this planet are currently facing. Since the issue of global crisis extends over the whole planet, it is required to assimilate the knowledge accumulated by the scientists in many fields. The Integrate Advanced Research Institute is constituted with the professors belongs to the three scientific faculties (Faculties of Science, Engineering, and Human Life Science) in the Sugimoto campus of Osaka City University. The first approved research project in this institute is the one that challenges the regeneration of the urban environment, and it tries to tackle the problems of biological systems and the sound and innovative use and circulation of energy and groundwater. The project is constituted with three strategic research subjects and is guided by three research teams as listed below.

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|---|---|
| Subject of Team A: | Development and Industrial Application of the Next Generation Energy Resources |
| Subject of Team B: | Utilization of Groundwater for the Preservation of Civil Environment and for the Prevention against Geo-hazard |
| Subject of Team C: | Space and Time Evolution of the Environment and Biological Systems in the Urban Area |

This international workshop is held on the subject of Team A. Eminent active researchers at home and abroad in the relevant research fields are invited as keynote speakers of this workshop. We hope that, through an animated discussion, we can share common realization of what should be done in the closest future in this field toward the resolution of worldwide affair.

Finally, I would like to express my sincere gratitude to Osaka City University for the generous financial support to establish this important workshop under the auspices of the specially distinguished advanced research project.

Hideki Hashimoto (Project Leader)

# International Workshop on Spectroscopic Studies of Carotenoids, Chlorophylls and Bacterial Photosynthesis

**DATE:** Jan 25th (Mon.), 2010 **SITE:** Kwansei Gakuin Kaikan, 1-1-155, Uegahara, Nishinomiya, Japan

**PROGRAM:**

| | | |
|---|---|---|
| 10:00 | Opening | Prof. Hideki Hashimoto |
| 10:05 | Prof. Yasushi Koyama (Kwansei Gakuin Univ. ) | "Excited-State Dynamics of Overlapped Optically-Allowed $1B_u^+$ and Optically-Forbidden $1B_u^-$ or $3A_g^-$ Vibronic Levels of Carotenoids: Possible Roles in the Light-Harvesting Function" |
| 10:45 | Break | |
| 10:55 | Prof. Hugo Scheer (Munich Univ., Germany) | "Hetero-Chlorophyllous Complexes of Peridinin-Chlorophyll-Protein: Reconstitution, Energy Transfer and Fluorescence Studies by Ensemble and Single Molecule Spectroscopy" |
| 11:20 | Prof. Peter Hannaford (Swinburne Univ. of Tech., Australia) | "Coherence Dynamics in Carotenoids by Two-Colour Spectrally-Resolved Transient Four-Wave Mixing" |
| 11:45 | Lunch Break | |
| 12:45 | Prof. Jian-Ping Zhang (Renmin Univ. of China, P. R. China) | "Synergistic Interaction between Carotenoid and Flavonoid in Antioxidation. Kinetics and Structure-Activity Relationship" |
| 13:10 | Prof. Heiko Lokstein (Univ. of Potsdam, Germany) | "Interactions of Xanthophylls and Chlorophylls in the Higher Plant Major Light-Harvesting Complex LHC II" |
| 13:35 | Break | |
| 13:45 | Prof. Hiroyoshi Nagae (Kobe City Univ. of Foreign Studies) | "Theoretical Aspects of Diabatic States and Their Mixing in Carotenoid Excited States" |
| 14:10 | Prof. Hideki Hashimoto (Osaka City Univ.) | "Sub-20 fs Coherent Spectroscopy of Carotenoids In and Out of Pigment-Protein Complexes" |
| 14:35 | Break | |
| 14:45 | Dr. Ritsuko Fujii (Osaka City Univ.) | "Pigment Composition in the Major Light-Harvesting Complexes from a Brown Alga, Okinawa Mozuku" |
| 15:05 | Dr. Leenawaty Limantara (Ma Chung Univ., Indonesia) | "A Tribute to Prof. Yasushi Koyama: Pioneer of Ma Chung Research Center for Photosynthetic Pigments" |
| 15:25 | Dr. Pu Qian (Shefield Univ., UK) | "Light Harvesting Complexes Facilitate Membrane Curvature in the Photosynthetic Bacterium *Rhodobacter sphaeroides*" |
| 15:45 | Break | |
| 15:55 | Dr. Tadashi Mizoguchi (Ritsumeikan Univ.) | "The Structure and Function of Long Esterifying Chains on (Bacterio)chlorophylls in Photosynthetic Antenna Systems" |
| 16:15 | Dr. Eiichi Nishizawa (Kao Brands Company) | "Wants" and "Thanks" |
| 16:35 | Dr. Xiao-Feng Wang (Gifu Univ., Japan) | "Highly Efficient Dye-Sensitized Solar Cells Based on Natural Chlorophylls and Their Derivatives" |
| 16:55 | Closing | Prof. Hideki Hashimoto (Osaka City Univ.)<br>Prof. Ruth M. Grubel (Chancellor of Kwansei Gakuin Univ.) |

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Dr. Ritsuko Fujii (Osaka City Univ., Japan) e-mail: ritsuko@sci.osaka-cu.ac.jp

**A part of this workshop is supported by the Integrated Advanced Research Institute of Osaka City University.**

# Excited-State Dynamics of Overlapped Optically-Allowed $1B_u^+$ and Optically-Forbidden $1B_u^-$ or $3A_g^-$ Vibronic Levels of Carotenoids: Possible Roles in the Light-Harvesting Function [1]

Yasushi Koyama [1,*], Yoshinori Kakitani [1], Takeshi Miki [1], Rebecca Christiana [1] and Hiroyoshi Nagae [2]

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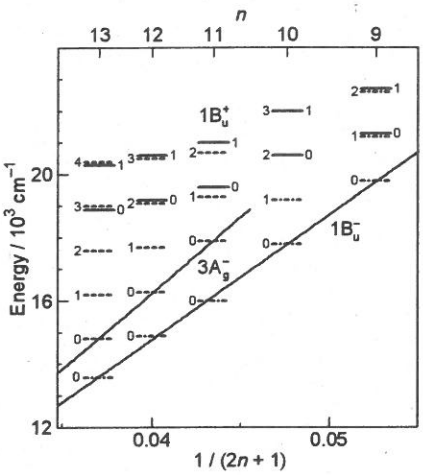


Pump-probe spectroscopy after *incoherent excitation* of all-*trans* Cars ($n$ = 9–13) in nonpolar solvent, probing stimulated emission and transient absorption, identified a symmetry selection rule of diabatic electronic mixing and diabatic internal conversion, i.e., '$1B_u^+$-to-$1B_u^-$ is allowed but $1B_u^+$-to-$3A_g^-$ is forbidden'. Kerr-gate fluorescence spectroscopy showed that this selection rule breaks down, due to the symmetry degradations when the Car molecules are being excited and, as a result, the $1B_u^+$-to-$3A_g^-$ diabatic electronic mixing and internal conversion become allowed.

On the other hand, pump-probe spectroscopy after *coherent excitation* in polar solvent identified three stimulated-emission components, generated by the quantum-beat mechanism, consisting of the long-lived coherent cross term from the $1B_u^+ + 1B_u^-$ or $1B_u^+ + 3A_g^-$ diabatic pair and incoherent short-lived $1B_u^+$ and $1B_u^-$ or $3A_g^-$ split incoherent terms. The same type of stimulated-emission components were identified in Cars bound to LH2 complexes, their lifetimes being substantially shortened by the Car-to-BChl singlet-energy transfer. The low-energy shifts of the $1B_u^+(0)$, $1B_u^-(0)$ and $3A_g^-(0)$ levels and efficient triplet generation were also found.

In all the above excited-state dynamics, the symmetry properties of the $1B_u^+$, $1B_u^-$ and $3A_g^-$ counterparts are totally conserved during the formation of the diabatic pairs and also during splitting and relaxation of the $1B_u^+$ counterpart through emission and that of the $1B_u^-$ or $3A_g^-$ counterpart through internal conversion. This is exactly what has been anticipated by the theoretical description (experimental condition) of the diabatic pairs.

The observed energetics and excited-state dynamics of the diabatic pairs and their rigorous theoretical description using the diabatic basis set fully support the symmetry notations, the energy diagrams and the potential curves for all the $1B_u^+$, $1B_u^-$, $3A_g^-$ and $2A_g^-$ vibronic levels we have been proposing.

[1] Y. Koyama *et al.*, to be submitted to *International Journal of Molecular Sciences*.

**Hetero-chlorophyllous complexes of peridinin-chlorophyll-protein: reconstitution, energy transfer and fluorescence studies by ensemble and single molecule spectroscopy**

S. Mackowski[1a], S. Wörmke[1], T.H.P. Brotosudarmo[1b], R.G. Hiller[2], E. Hofmann[3], C. Bräuchle[1], H. Scheer[4*]

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Successful reconstitution of the N-terminal domain apoprotein of the peridinin-chlorophyll-protein (PCP) complex with mixtures of chlorophylls (Chl) absorbing at different wavelengths offers a possibility to study energy transfer processes between the two Chls in this system.

By selecting Chl pairs with suitable spectral properties, such as absorption maximum, extinction coefficient, fluorescence lifetime and quantum yield, various regimes of the energy transfer can be probed in such hetero-chlorophyllous complexes. Homo- and heterochlorophyllous complexes were prepared with three Chls that differ mainly in the energies of their long-wavelength absorption bands, viz. Chl a, Chl b and [3-acetyl]-Chl a. Affinities of the different chlorophylls to the complex were determined by pigment analysis of the reconstituted complexes. Surprisingly, binding of the natural pigment, Chl a, is only half as strong as binding of Chl b or [3-acetyl]-Chl a.

We report on detailed ensemble and single molecule studies [1] of N-PCP reconstituted with binary mixtures of Chl a, Chl b and [3-acetyl]-Chl a. Single molecule spectroscopy provides a clear distinction between individual homo- and hetero-chlorophyllous complexes. Energy transfer is bidirectional for samples containing one Chl a and one Chl b within a monomer, corresponding to an energy gap of ~20 nm. It becomes unidirectional in complexes that contain one Chl b and one [3-acetyl]-Chl a (energy gap ~40 nm), or one Chl a and one [3-acetyl]-Chl a. The results are analyzed using Förster theory energy transfer combined with Monte Carlo simulations.

We also report on fluorescence enhancement by nearly an order of magnitude of single PCP complexes deposited on nanostructured metal surfaces [2].

[1] S. Wörmke, S. Mackowski, T.H.P. Brotosudarmo, and Ch. Bräuchle, A. Garcia, P. Braun, and H. Scheer, E. Hofmann (2007) Appl. Phys. Lett. 90, 193901.
[2] S. Mackowski, S. Wörmke, A.J. Maier, T.H.P. Brotosudarmo, H. Harutyunyan, A. Hartschuh, A.O.Gogorov, H. Scheer, C. Bräuchle (2008) Nano Lett. 8, 558.

## Coherence Dynamics in Carotenoids by Two-Colour Spectrally-Resolved Transient Four-Wave Mixing

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We report studies of the coherence dynamics in excited states of lycopene and spheroidene in solution using two-colour, three-pulse, spectrally resolved transient four-wave mixing (FWM) spectroscopy [1]. The first two pulses which have different colours are tuned to create a coherent superposition of two excited states and the third pulse creates a third-order polarization signal in the phase-matched direction which is analysed in a spectrometer. A non-interferometric 2D Fourier transform technique [2] is used to retrieve the phase from the spectral intensity measurements and hence to determine the full temporal evolution of the complex third-order polarization, thus enabling the determination of 2D correlation spectra of the detection frequency versus excitation frequency. The advantage of this technique compared to direct determination of the phase by heterodyne methods is that it is readily applicable to two-colour FWM experiments.

In our two-colour FWM experiments pulse 1 is tuned to the bright transition $1A_g^-(0) \rightarrow 1B_u^+(0)$ in lycopene and pulses 2 and 3 are tuned to identify states that are vibronically coupled to the $1B_u^+(0)$ state. When pulses 2 and 3 are resonant with with transitions to the $2A_g^-(4)$ dark state, the time-resolved FWM signal extends out as far as 2 ps, indicating coherent coupling between the very short-lived $1B_u^+(0)$ bright state and the very long-lived $2A_g^-(4)$ dark state [3]. Similar long-lived quantum coherences are found for the corresponding states in spheroidene. Recent experimental evidence elsewhere [4] suggests that coherence transfer processes may play a significant role in the highly efficient energy transfer in light-harvesting complexes. Our results indicate that long-lived coherent quantum coupling can also occur in single light-harvesting molecules in solution within isolated chromophores.

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[2] J.A. Davis *et al.*, *Phys. Rev. Lett.* **100**, 227401 (2008).
[3] J.A. Davis *et al.*, submitted for publication.
[4] G.S. Engel *et al.*, *Nature* **446**, 782 (2007).

## Synergistic Interaction between Carotenoid and Flavonoid in Antioxidation. Kinetics and Structure-Activity Relationship

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Carotenoids and flavonoids presenting ubiquitously in the plant kingdom are transferred through the food chain to animals to carry out specific biological functions. In relation to human health, numerous epidemiological researches have shown that a high intake of flavonoids [1] or carotenoids [2] via consumption of fruits and vegetables is health beneficial, which is often correlated to the antioxidant activities of these natural compounds. Despite the extensive investigations on the individual groups of carotenoids or flavonoids as antioxidants, the possible synergism between them have not been studied in any details although there are some indications of electron transfer between $\beta$-carotene radical cations formed from oxidative stress and puerarin or baicalin at water/lipid interfaces. This report presents the recent results on the synergistic carotenoid-flavonoid interactions in model systems with emphasis on the structure-activity relationship and the related free radical reaction kinetics [3, 4].

[1] P. C. H. Hollman, M. B. Katan, *Food Chem. Toxicol.* **37**, 937 (1999).

[2] R. Edge, D. J. McGarvey, T. G Truscott, *J. Photochem. Photobiol.* **41**, 189 (1997).

[3] J.-P. Zhang *et al.*, *J. Agric. Food. Chem.* **55**, 2384 (2007); **56**, 10376 (2008); **57**, 19 (2009); **57**, 3780 (2009).

[4] J. Liang, Y.-X. Tian, F. Yang, J.-P. Zhang, L. H. Skibsted, *Food Chem.* **115**, 1437 (2009).

**Interactions of xanthophylls and chlorophylls in the higher plant major light-harvesting complex LHC II**

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Besides chlorophylls (Chls) *a* and *b* plant main light-harvesting complex (LHC II) binds xanthophylls - two luteins, neoxanthin, violaxanthin and/or its de-epoxidation products per monmeric subunit. These xanthophylls fullfil several important functions in LHCs: structure stabilization, light-harvesting and photoprotection. LHC II, in vitro as well as in vivo, can exist in various states of aggregation profoundly altering interactions between pigments. Aggregation of LHC II *in vitro* is thought to mimic structural changes that may be the basis of the photoprotective process assessed as the energization-dependent component (qE) of nonphotochemical quenching of Chl fluorescence (NPQ). The so-called xanthophyll cycle (light-stress induced, dark-reversible, de-epoxidation of violaxanthin via anthraxanthin to zexanthin) appears to be crucial in this regard, too. The exact molecular mechanism of xanthophyll involvement in qE/NPQ has not been elucidated, yet. Recent proposals regarding the qE mechanism(s) will be reviewed. Nonlinear polarization spectroscopy in the frequency domain (NLPF, *cf.* [1,2]) was used to investigate the changes in the interactions between xanthophylls and Chls in spinach LHC II upon alteration of its aggregation state by incubation at different detergent concentrations. NLPF spectra of slightly aggregated and trimeric LHC II were measured - pumping in the Chl-$Q_y$ region and probing in the xanthophyll $2^1B_u^+$- region. NLPF spectra of trimeric LHC II are dominated by a peak at about 652 nm (due to Chl *b*) - with virtually no contribution from Chl *a*. For LHC II aggregates the spectra peak in the Chl *a* $Q_y$ region (at about 682 nm). These changes are discussed with regard to alterations in the interactions of xanthophyll molecule(s) being in intimate contact with several Chls *b* in trimeric LHC II. Additionally, two-photon excitation with tunable 100 fs-laser pulses was used to measure the xanthophylls $1^1A_g^- \rightarrow 2^1A_g^-$ transition profiles in LHC II samples containing different xanthophyll complements. Implications of the results for the mechanism(s) of NPQ will be discussed.

*Acknowledgements*: This research is supported by the Deutsche Forschungsgemeinschaft (Collaborative Research Center SFB 429).

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## Theoretical aspects of diabatic states and their mixing in carotenoid excited states

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Based on the measurement of resonance-Raman excitation profiles, a pair of new formally forbidden states ($3Ag^-$ and $1Bu^-$) was identified, in addition to the well-accepted optically-active $1Bu^+$ and formally forbidden $2Ag^-$ state [1]. Because of the unique linear relations for the $1Bu^+(0)$, $1Bu^-(0)$ and $3Ag^-(0)$ origins, the $1Bu^+(0)$ level approximately match the $1Bu^-(1)$ and $1Bu^-(2)$ levels in Car($n$ = 9 and 10), while $3Ag^-(1)$, $3Ag^-(2)$ and $3Ag^-(3)$ levels in Car($n$ = 11 ,12 and 13).

When two potential surfaces approach within the distance of the relevant vibrational energy in the region where the probability density of nuclear coordinates is high, the Born-Oppenheimer or adiabatic approximation breaks down, and quasi-diabatic description will be needed. Thus, carotenoid $1Bu^+$, $1Bu^-$ and $3Ag^-$ excited states should be described by using the quasi-diabatic basis [2]. It can be shown that the quasi-diabatic basis of low-lying excited states of carotenoids can be described by wavefunctions with constant MO and CI coefficients and with floating orthogonalized atomic orbitals.

Based on the diabatic theory,

(i) electronic mixing and internal conversion between the diabatic $1Bu^+$, $1Bu^-$ and $3Ag^-$ excited states are examined in relation to the experimentally-found selection rule for state mixing.

(ii) diabatic mixing and coherent excitation of a pair of the diabatic states are described to explain the oscillatory changes in transient absorption or fluorescence intensity (quantum beat).

(iii) life time of the diabatic mixing states is examined to explain the appearance of longer-lived excited states of carotenoids.

[1] Y. Koyama, Y. Kakitani, Y. Watanabe, in: G. Renger (Ed.), Primary Processes of Photosynthesis, Part 1: Principles and Apparatus, RSC Publishing, Cambridge, 2008, Chap. 4.

[2] T. Pacher, L.S. Cederbaum and H. Köppel. Adv. Chem. Phys. 84 (1993), p. 293.

## Sub-20 fs Coherent Spectroscopy of Carotenoids In and Out of Pigment-Protein Complexes

Hideki Hashimoto [1*], Ritsuko Fujii [1], Richard J. Cogdell [2], and Mitsuru Sugisaki [1]

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The primary process of photosynthesis starts from the capture of sunlight by light-harvesting complexes and then followed by excitation-energy transfer to reaction centres. The electronic and vibronic structures of concomitant photosynthetic pigments in and out of these pigment-protein complexes have attracted considerable attention in order to reveal the detailed mechanisms of the highly efficient energy-transfer. This lecture mainly focuses on the photophysical properties of carotenoids in the light-harvesting system. In spite of a great deal of experimental data, there is still a great deal of confusion in this area of carotenoid science. This lecture will provide a careful description of what we know, what we don't know and why there are still outstanding problems. The classic description of carotenoid photophysics is largely based on the theoretical framework provided many years ago by Tavan and Schulten [1,2]. Their calculations introduced the idea that there were two well-defined excited singlet states called $S_1$ ($2^1A_g^-$) and $S_2$ ($1^1B_u^+$). Although $S_1$ is the lowest excited singlet state, because it cannot formally be populated by a one-photon excitation, the lowest one-photon allowed excited singlet state is $S_2$. The transition from the ground ($1^1A_g^-$) state to the $S_2$ state is responsible for the well-known, strong visible absorption bands of carotenoids. The problem of understanding the details of carotenoid photophysics comes not only from this basic complicated picture but also from the fact that the $S_1$ and $S_2$ states have very short lifetimes. Moreover, as faster and faster spectroscopic techniques have been applied to the study of carotenoids, more difficult to characterize 'states' have been described [3-5]. There is now not only disagreement in the field about the data, but also about how to assign whether these 'new' states are due to new electronic states, vibrational states or changes in the conformation of carotenoids in the excited states. This lecture will explore these points and try to outline ways in which the existing data can be reconciled to produce a consistent picture, even if that is only to pin-point where we disagree.

[1] P. Tavan and K. Schulten, *J. Chem. Phys.* **85**, 6602 (1986).
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[4] T. Polívka and V. Sundström, *Chem. Rev.* **104**, 2021 (2004).
[5] T. Polívka and V. Sundström, *Chem. Phys. Lett.* (2009) in press.

# Pigment Composition in the Major Light-Harvesting Complexes from a Brown Alga, Okinawa Mozuku

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More than a half of photosynthesis is performed in the ocean, although the oceanic photosynthesis is relatively less studied [1, 2]. *Cladosiphon okamuranus* is an original strain of an edible brown alga in Okinawa, called Okinawa Mozuku in Japanese, which have fucoxanthin-chlorophyll *a*/*c* protein (FCP) as a major photosynthetic antenna complex. FCP is thought to transfer energy to both oxygenic photosystem I and II, although the details are not clarified yet. Recently Iha et al. has been successful to culture a discoid germiling of the Okinawa Mozuku, which is a precursor of an alga body. We have established the isolation and purification method of the FCP from the discoid germiling. We also have found the FCP exists as a homo- or hetero-trimmer formed by the products of three highly homologous subunits in unequal stoichiometries. Hereafter, we call this particular complex as 'Mozuku FCP'. Our future goal is to clarify the 3D-structure of this 'Mozuku FCP' by using X-ray crystallography. In this study, we determined the optical properties of the pigments bound to the 'Mozuku FCP', which has high biochemical degree of purity. First, we determined the pigment composition of the 'Mozuku FCP' using HPLC and $^1$H-NMR. The FCP has been reported to contain fucoxanthin, chlorophyll (Chl) *a* and Chl $c_2$ in the molecular ratio of 4~6 : 4 : 1, respectively [3-7]. Despite those reports, we for the first time found that the 'Mozuku FCP' contains Chl $c_1$ and Chl $c_2$ in almost the same composition. Then, we also measured the fluorescence and fluorescence-excitation spectra of the 'Mozuku FCP' to determine the efficiencies of energy transfer from both fucoxanthin and Chl *c* to Chl *a*.

## References

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# A Tribute to Prof. Yasushi Koyama: Pioneer of Ma Chung Research Center for Photosynthetic Pigments

**Leenawaty Limantara**

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What the writer is going to present on the International Workshop on Prof. Koyama's Mandatory Retirement is a token of deepest gratitude to Prof. Koyama for his wise guidance and friendship commencing since 1991 up to now. The comment will be focused on the initiation aspect and development of the University the writer has led, that is Ma Chung University, Malang, East Java, Indonesia (www.machung.ac.id) and the research development through Ma Chung Research Center For Photosynthetic Pigments (MRCCP, www.machung.ac.id/mrccp) which Prof. Yasushi Koyama has significantly inspired.

It is easy to start off an idea and turn it into an organization, but the most difficult part of it is to develop and maintain the organization so that it achieves world scale level and quality. Since 2007 up to now, Ma Chung University and MRCCP are not three years old yet, and yet a variety of values, vision, strategies, and work programs have been conducted to give a special colour to the university, to show its identity, and to bring it to the glorious ideals of its founders, such as producing graduates with lofty characters and responsible future leaders in any environment they are established.

MRCCP, starting its activities in 2008, was born on account of the enthusiasm and continuous encouragement from world class scientists such as Prof. Koyama, Prof. Scheer, and Prof. Cogdell. They are unique scientists, they are in fact contradicting each other. The writer has known them since 1990-ish and they serve as role models and sources of inspiration for the writer while she is leading her life as Rector and researcher. The writer presents this writing as her tribute to Prof. Koyama so that he will be relieved and proud to know that his painstaking efforts in teaching and educating his students do not only produce reliable and excellent researchers, but also a university leader.

# Light Harvesting Complexes Facilitate Membrane Curvature in the Photosynthetic Bacterium *Rhodobacter sphaeroides*

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In the photosynthetic purple bacterium *Rhodobacter sphaeroides*, two different light harvesting (LH) complexes LH2 and LH1 are accommodated into lipid bi-layer membrane. As an analog of the LH2 complex from *Rhodopseudomonas acidophila*(1), LH2 from *Rb. sphaeroides* has nona-symmetric ring-like structure as well (2). The LH1 core complex, however, is very unique in this species, taking a dimeric form due to the existence of an extra trans-membrane polypeptide, called PufX, i.e., $(RC\text{-}LH1\text{-}PufX)_2$ (3). Both LH2 and LH1 complex exhibits an intrinsic character inducing curved membrane. The membrane from LH2 only mutant revealed by high resolution AFM shows that membrane takes spherical shape, indicating LH2 itself has ability to facilitate membrane curvature (4). This is consistent with the results from molecular dynamic simulation (5). In the case of LH1 only mutant, tubular membrane, consisting of dimeric core complex can be seen. Electron microscopy single particle analysis of dimeric core complex revealed that the two haves of the dimer molecule incline toward each other on the periplasmic side, creating a remarkable V-shape structure (6). Obviously, it is the bending dimer molecules that results in the formation of tubular membrane. Computer simulation (MDFF) showed how the dimer produces a membrane with high local curvature (7). The resulting membrane curvature agrees well with the observed size using electron microscope. Combined all structure information available, a model of spherical membrane from wilt type *Rb. sphaeroides* is built up.

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# The Structure and Function of Long Esterifying Chains on (Bacterio)chlorophylls in Photosynthetic Antenna Systems

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Almost all naturally occurring chlorophyll (Chl) pigments have a propionate-type ester group at the $17^2$-position (17-$CH_2$–$CH_2$–$CO_2$-R; R = phytyl etc.) except most Chls-*c*. In general, Chl-*a* found in oxygenic phototrophs and bacteriochlorophyll (BChl)-*a* in anoxygenic photosynthetic bacteria have a phytyl group as their ester group [1,2]. Such (B)Chl molecules are biosynthesized in photosynthetic organisms and function in light-harvesting (LH) and reaction center (RC) complexes. The esterifying substituent is not directly conjugated with the π-system in (B)Chl molecules and does not affect the electronic-absorption spectra of their monomeric states. As a result, the moiety has attracted less attention in comparison with other peripheral substituents, although it constitutes about 30 - 40% of the weight of a molecule. The hydrophobic propionate-type ester group is believed to play an important role in stabilizing complexation with peptides in photosynthetic apparatus. Moreover, it serves as an anchor to lock in (B)Chl molecules at the appropriate position in pigment-protein complexes. Recent progress in crystallization of pigment-protein complexes enables us to determine the structure of apo-proteins as well as that of photosynthetic pigments at an atomic level [3]. However, the ester group often showed low density of the diffraction in X-ray analysis primarily due to its flexibility and, as a result, the structure and/or the detailed binding mechanism to photosynthetic peptides remained unclear. In this report, we investigated the multiplicity and accumulation of (B)Chl molecules having various 17-propionate ester groups in photosynthetic organisms. Furthermore, we isolated pigment-protein complexes including LH2, LH4 and LH1-RC in order to determine the preferential accumulation of such (B)Chl molecules on the pigment-protein complexes. These results enable us to discuss a role of the hydrophobic ester group on construction and regulation of pigment-protein complexes in photosynthetic antenna systems.

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# "Wants" and "Thanks"

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"Wants" and "Thanks" are most important things for the excellent study. This is what I learned from Prof. Koyama. For 6 years under his education, I had studied hard about the excited states of chlorophylls by using the time-resolved resonance Raman and absorption spectroscopy. His "Wants" to elucidate the mystery of photosynthesis, attracted me and made me fall into the study on the primary photosynthesis. I had perceived the "Wants" is the origin of power to clear many hurdles. Then I also "Thanked" nature to charm me and giving me strong motivation. I've really "Thanked" my companions as well. Through the cross-functional study with them having a different knowledge and culture, we helped each other and encouraged to reach our common goal together.

Apart from the original field, I am now studying to develop hair coloring and styling products. In the same way, "Wants" and "Thanks" play both important roles in the creation of "Innovative Technology", "Nobel Formulas" and "Excellent Consumer Products". "Wants" to understand more my consumer, to develop innovative technology and to provide new essential benefits with wholehearted satisfaction through consumer products. "Thanks" for consumer to fascinate me, for coworker to cross-functionally work together and for science to bring us enrichment of their lives.

On the consumer product development, I always keep in my mind that it is important to describe clearly "Consumer Wants", "Innovative Technology" and "Essential Benefits" for the creation of the consumer products.

I will introduce my experience of the product development in accordance with the above. The topic is "The selective penetration enhancing system for acid dyes [1]" regarding hair color product with non-oxidation coloring. This study was conducted on corresponding to "Consumer Wants"; *"More hair dyeing and Less skin staining"*. We could succeed to create a new product with innovative technology, the selective dye-penetration system. This technology was invented by the cross-functional study on the different mechanisms of hair dyeing and skin staining.

I'd like to continue studying with "Wants" and "Thanks".

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## Highly Efficient Dye-Sensitized Solar Cells Based on Natural Chlorophylls and Their Derivatives

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Replacing traditional fossil fuels as power supply with renewable energy is a global consensus. However, the process of producing renewable energy is not always 'green'. For example, manufacturing Si-based solar cells needs lots of energy, and releases large amount of $CO_2$ to the atmosphere. Dye-sensitized solar cells (DSSCs) are alternatives of such Si-based solar cells, because they are much 'green' in fabrication. In DSSCs, a dye sensitizer plays a key role in the light-harvesting and the electron-transferring processes. Dye sensitizers based on cyclic tetrapyrroles, especially chlorophylls, are well known for their important functions in photosynthesis. Natural photosynthesis has been evolved over billions of years with adaptation of gradually changed Earth's climate and environment. Apparatus in the photosynthetic initial stage mainly require elements of H, C, N, O, Mg, and Fe, which are easily available and reusable. The philosophy behind the present research is to develop DSSCs by using chlorophyllous dyes as a 'gift' from natural photosynthesis, to minimize risks that may come from the synthetic processes or environmental contamination as well as to maximize utilization efficiency of solar energy including lights in the near-infrared region. In the present study, we will focus on to establish a relationship between the molecular structure of chlorophyllous sensitizer and the performance of DSSC. The highest solar energy-to-electricity conversion efficiency of up to 8% has been achieved.