COST PHOTOTECH Training School

Advanced Laser Spectroscopy in Green Phototechnology

Program and Book of Abstracts

October 18-23, 2014

Szeged, Hungary

Sponsors

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COST TD1102 (PHOTOTECH)

European Biophysical Societies' Association (EBSA)

International Society of Photosynthesis Research (ISPR)

Biological Research Center, Hungarian Academy of Sciences, Szeged

University of Szeged

Hungarian Biophysical Society

"Life from Light" - Photosynthesis Foundation

City of Szeged

Welcome

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PROGRAM

October 18 (Saturday)

14:00-17:30 **REGISTRATION**
(*Art Hotel*)

University of Szeged – Rector's Office, Assembly Hall

17:30-18:00 **OPENING ADDRESSES**

Gábor Szabó, Rector of the University of Szeged

Pál Ormos, Director General of the Biological Research Center, Szeged

László Botka, Mayor of Szeged

Giuseppina Rea, Chair of COST Action PHOTOTECH

Béla Rácz, Head of TÁMOP Program

COST PHOTOTECH / ELI-ALPS

Chair: ***Gábor Szabó***

18:00-18:30 **Giuseppina Rea** – PHOTOTECH: optobioelectronic devices to study photosynthesis, design biosensors and harness solar energy

18:30-19:00 **Lóránt Lehrner** – The status of ELI-ALPS project

19:00-19:30 **Dimitris Charalambidis** – Studies of ultrafast dynamics by non-linear XUV processes & their prospects at ELI-ALPS

University of Szeged – Rector's Office, Inner Courtyard

19:30-22:00 **WELCOME RECEPTION**
(Short Concert of Kosztándi String Quartet and Buffet Dinner)

October 19 (Sunday)

Szeged Regional Academy Building (SZAB)

PRIMARY PROCESSES OF PHOTOSYNTHESIS

Chair: ***Péter Maróti***

09:00-09:30 **Rienk van Grondelle** – The quantum design of photosynthesis

9:30-10:00 **Richard Cogdell** – How to investigate the structure and function of light harvesting complexes

10:00-10:30 **Roberta Croce** – Light harvesting in the green alga *Chlamydomonas reinhardtii*

10:30-11:00 **COFFEE BREAK**

XFEL. PHOTOSYNTHETIC PROTEIN COMPLEXES. I

Chair: ***Pál Ormos***

11:00-11:30 **Gyula Faigel** – New uses of an old method, the x-ray diffraction

11:30-12:15 **János Hajdú** – Imaging experiments with free electron lasers

12:15-12:45 **Jan Kern** – Watching photosystem II at work using fs X-ray pulses

12:45-14:30 **LUNCH**

(*Art Hotel*)

Szeged Regional Academy Building (SZAB)

XFEL. PHOTOSYNTHETIC PROTEIN COMPLEXES. II

Chair: ***György Dombi***

14:30-15:00 **Mike Jones** – The best of both worlds: SMALPs for solubilised photosynthetic membrane proteins with native-like properties

15:00-15:30 **Huub de Groot** – Responsive matrices for solar to fuel conversion: trading time for efficiency

15:30-16:00 **Thomas Renger** – Building principles of photosynthetic light-harvesting antennae: Theory meets experiment

16:00-16:30 **Fabio Polticelli** – 21st century challenges at the interface of biology and computer science

16:30-17:00 **COFFEE BREAK**

Szeged Regional Academy Building (SZAB)

VIBRATIONAL DYNAMICS AND RAMAN SPECTROSCOPY

Chair: ***Balázs Szalontai***

17:00-17:30 **Bruno Robert** – Molecular basis of photoprotection in natural and artificial photosynthesis

17:30-18:00 **Miroslav Kloz** – Femtosecond stimulated Raman spectroscopy in 1D and 2D – basics and beyond

18:00-18:30 **Yang Zhao** – Davydov states for energy transfer dynamics in light harvesting systems

18:30-20:30 **DINNER**

(*Art Hotel*)

Art Hotel – Somogyi Lecture Hall

TERAHERTZ SPECTROSCOPY

Chair: ***László Zimányi***

20:30-21:00 **János Hebling** – Terahertz measurement technology in photobiophysics and at ELI-ALPS

21:00-21:20 **József Orbán** – THz vibrational study of biomolecules

October 20 (Monday)

Szeged Regional Academy Building (SZAB)

LIGHT HARVESTING COMPLEXES. I

Chair: ***Győző Garab***

09:00-09:30 **Alfred R. Holzwarth** – Light-harvesting, antenna quenching, and ultrafast charge separation in reaction centers. A unified view

09:30-10:00 **Herbert van Amerongen** – Photosynthetic complexes studied with picosecond spectroscopy: from dead crystals to living cells

10:00-10:30 **Arvi Freiberg** – "Biological variability" in the research of photosynthetic light harvesting

10:30-11:00 **COFFEE BREAK**

LIGHT HARVESTING COMPLEXES. II

Chair: ***Béla Hopp***

11:00-11:30 **Tomas Polivka** – Carotenoids in regulation of energy flow through photosynthetic antenna – Light harvesting vs. photoprotection

11:30-12:00 **Leonas Valkunas** – Distinctive character of electronic and vibrational coherences in photosynthetic pigment-protein complexes

12:00-12:30 **Howe-Siang Tan** – Ultrafast multi-dimensional electronic spectroscopy and its applications to the study of LHCII

12:30-12:45 **Andrius Gelžinis** – Mapping energy transfer flow in fucoxanthin-chlorophyll protein complex

12:45-14:30 **LUNCH**

(*Art Hotel*)

Szeged Regional Academy Building (SZAB)

LIGHT HARVESTING COMPLEXES. III

Chair: ***László Nagy***

14:30-14:50 **Leszek Fiedor** – Metallosubstituted (bacterio)chlorophylls – Ultrafast molecular devices for studies of photosynthetic processes in time and space

14:50-15:10 **Krzysztof Gibasiewicz** – Protein dynamics as a factor influencing the intraprotein electron transfer

15:10-15:30 **Raoul Frese** – Biosolar cells: natural and artificial assemblies of light energy transducing protein complexes

15:30-15:50 **Petar H. Lambrev** – Pigment interactions in light-harvesting complex II in different molecular environments

15:50-16:05 **Franz-Josef Schmitt** – Efficient energy harvesting with nanostructured hybrid systems containing photosynthetic pigment-protein-complexes

16:05-16:20 **Jevgenij Chmeliov** – Light harvesting in a fluctuating antenna

16:20-18:00 **POSTER SESSION AND COFFEE BREAK**

18:00-20:00 **DINNER**

(*Art Hotel*)

20:00-21:00 **CULTURAL PROGRAM**

(Concert of Premier Trombone Quartet, *REÖK Palace*)

October 21 (Tuesday)

Szeged Regional Academy Building (SZAB)

ULTRAFAST PHOTOBIOLOGICAL PROCESSES

Chair: ***Klára Hernádi***

09:00-09:25 **András Dér** – Protein-based high speed all-optical logic

09:25-09:50 **Géza Groma** – Hofmeister effect on the FAD molecule revealed by fluorescence lifetime analysis in the fs-ns range

09:50-10:15 **András Lukács** – Functional dynamics of BLUF domain proteins revealed by ultrafast spectroscopy

10:15-10:40 **Nicolas Plumeré** – Photosynthetic protein in biophotovoltaics - the issue of charge recombination

10:40-11:05 **Károly Osvay** – Ultrafast light pulse sources for time resolved studies of ELI-ALPS

11:05-11:30 **COFFEE BREAK**

MONITORING CHARGE MOVEMENTS

Chair: ***Imre Vass***

11:30-12:00 **Michael Haumann** – Advanced X-ray spectroscopy methods for characterization of small-molecule activating transition-metal centers in biological enzymes and catalytic materials

12:00-12:30 **Nicholas Cox** – The structure of nature's water splitting catalyst prior to O-O bond formation: Water binding and water splitting in photosynthesis

12:30-13:00 **Giuseppe Sansone** – Electron dynamics in molecules investigated by attosecond pulses

13:00-13:10 **CLOSING REMARKS**

13:10-14:30 **LUNCH**

(*Art Hotel*)

14:30-18:30 **Laboratory Practicals (*see on the next page*)**

18:30- **Free Evening**

October 22 (Wednesday)

09:00-13:00 **Laboratory Practicals***

13:00-14:30 **LUNCH**
(*close to the site of the practical*)

14:30-18:30 **Laboratory Practicals**

18:30- **Free Evening**

Laboratory Practicals (places, titles, supervisors):

1) Place: Laboratory of TEWATI, Department of Optics and Quantumelectronics, University of Szeged, Dóm tér 9.
Title: Measuring primary charge separation in photosynthetic reaction centers by pump-probe transient absorption
Teachers: Ádám Börzsönyi, Roland Flender, Károly Osvay

2) Place: Department of Medical Physics and Informatics, University of Szeged, Rerrich Béla tér 1. (Béke building) 3rd floor
Title: Measuring electric signals of photosynthetic reaction centers-nanohybrid systems in dried conditions
Teacher: Tibor Szabó

3) Place: Institute of Plant Biology, Biological Research Center, Labs. 113 and 019, Szeged, Temesvári krt 62.
Title: Molecular characterisation of photosynthetic complexes by circular dichrosim and time-resolved fluorescence spectroscopy
Teachers: Petar Lambrev, László Kovács

4) Place: Institute of Biophysics, Biological Research Center Lab 342 (3rd floor), Szeged, Temesvári krt 62.
Title: Protein based, high speed integrated optical logic
Teachers: Sándor Valkai, Anna Mathesz

5) Place: Institute of Biophysics, Biological Research Center, Room 388 and Lab 344 (3rd floor), Szeged, Temesvári krt 62.
Title: Femtosecond Time-Resolved Fluorescence Spectroscopy
Teachers: Géza Groma, Ferenc Sarlós

October 23 (Thursday)

Trainees' presentations and discussions

Art Hotel – Somogyi Lecture Hall

PRIMARY PROCESSES OF PHOTOSYNTHESIS

Chairs: ***Arvi Freiberg and Jan Kern***

09:00-09:20 **Shazia Farooq** – Study of excited-state kinetics of photosystem I and II in intact spinach leaves by picosecond fluorescence lifetime measurements

09:20-09:40 **Parveen Akhtar** – Effects of detergents, lipids and trimer-trimer contacts on the pigment excitonic interactions in plant light-harvesting complex II (***see also poster 10***)

09:40-10:00 **Dominik Lindorfer** – Towards a structure-based exciton Hamiltonian for the CP29 antenna of photosystem II (***see also poster 4***)

10:00-10:20 **Reza Ranjbar Choubeh –** Design principles and efficiency of light harvesting complexes in green bacteria. A picosecond fluorescence study.

10:20-10:40 **Wojciech Giera** – Excitation dynamics in Photosystem I studied by streak-camera measurements at room temperature and at 77 K (***see also poster 12***)

10:40-11:00 **Elizabeth Kish** – Peridinin and Carbonyl Fermi Resonance, A Biophysical Study using Resonance Raman Spectroscopy (***see also poster 5***)

11:00-11:20 **COFFEE BREAK**

HYBRID SYSTEMS

Chairs: ***Howe-Siang Tan and Mike Jones***

11:20-11:40 **Marianneza Chatzipetrou** – Laser Induced Forward Transfer as an immobilization technique, for biomaterials (***see also poster 13***)

11:40-12:00 **Melania Kujawa** – Interaction between Rhodobacter sphaeroides reaction centers and TiO_2

12:00-12:20 **Nicholas Paul** – Ultrafast Quenching of Chlorophyll Excitons in Photosystem II Antenna Coupled to Indium Tin Oxide Nanoparticles (***see also poster 9***)

12:20-12:40 **Zsuzsanna Heiner** – Investigation of plasmonic nanostructures for multiphoton spectroscopic applications based on pH-sensing

12:40-14:00 **LUNCH**

(*Art Hotel*)

MISCELLANEOUS

Chairs: ***Fabio Polticelli and László Nagy***

14:00-14:20 **Violeta Peeva** – Photosystem II thermoluminescence and oxygen evolving activity in isoprene-emitting and non-emitting tobacco leaves and isolated thylakoids before and after UV-B irradiation

14:20-14:40 **Kathleen Feilke** – In vitro analysis of the plastid terminal oxidase in photosynthetic electron transport

14:40-15:00 **Rabail Razzaq** – Removal Of Ni(II) From Aqueous Solutions By Strong Cation Exchange Resin, Amberlyst-15(H^+)

15:00-15:20 **Krisztina Nagy** – Microfluidic gradient generators for measuring bacterial chemotactic response

15:20-15:40 **COFFEE BREAK**

SOLAR CELLS

Chairs: ***Giuseppina Rea and Krzysztof Gibasiewicz***

15:40-16:00 **Kumud B. Mishra** – Fluorescence based methods for selection of highly efficient photosynthetic apparatus for bio-sensors/biochips application

16:00-16:20 **Rafał Białek** – Construction of Solar Cells Based on *Rhodobacter sphaeroides* Reaction Centers and TiO_2

16:20-16:40 **Ambra Guarnaccio** – A new triad donor-acceptor compound: synthesis and photophysical behaviour

16:40-17:00 **Ntevhe Thovhogi** – Transport Properties Of Self-Assembled Porrphyrins & C60 Nanorods

17:00-17:20 **Jianjun Wang** – Functionalised hematite photoelectrode with engineered C-phycocyanin for hydrogen generation by water splitting (***see also poster 6***)

19:00-21:00 **CLOSING CEREMONY / DINNER**

LECTURES

PHOTOTECH: optobioelectronic devices to study photosynthesis, design biosensors and harness solar energy

G. REA

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The photosynthetic machinery is a smart assembly of *ad hoc* light collectors, protein-metal clusters, and redox biocatalysts enabling the conversion of solar energy into chemical energy. The process relays on the transduction of photo-excitation events into transmembrane charge-separated states that occurs with very high quantum efficiency, and a series of electron transfer reactions leading to the production of all the goods that fuels our daily life.

The COST Action TD1102 PHOTOTECH Photosynthetic proteins for technological applications: biosensors and biochips, operating through a network of researchers and professionals that can combine and integrate diverse disciplines, represents a good opportunity for carrying out and implementing an interdisciplinary approach to the study of the theoretical and practical aspects of photosynthesis relevant for the co-development of a fully characterized class of bio-organic-inorganic hybrids for biosensors and bio-energy production. The purpose is to find innovative and scientifically sound solutions to critical issues hindering the wide development and use of photosynthesis-based optoelectronic devices.

Acknowledgement
I acknowledge all participants contributing to the success of PHOTOTECH. PHOTOTECH is funded by COST. COST is Europe's longest-running intergovernmental framework for cooperation in science and technology funding cooperative scientific projects called 'COST Actions'. With a successful history of implementing scientific networking projects for over 40 years, COST offers scientists the opportunity to embark upon bottom-up, multidisciplinary and collaborative networks across all science and technology domains.

The status of ELI-ALPS project

L. LEHRNER

Studies of ultrafast fast dynamics by non-linear XUV processes & their prospects at ELI-ALPS

D. CHARALAMBIDIS

Department of Physics, University of Crete, PO Box 2208, GR71003 Heraklion (Crete), Greece Foundation for Research and Technology - Hellas, Institute of Electronic Structure & Laser, PO Box 1527, GR71110 Heraklion (Crete), Greece, chara@iesl.forth.gr

In the last 15 years we have systematically developed advanced sources of energetic XUV pulses with 1fs to sub-fs pulse duration, based on the process of higher order harmonic generation (HOHG). Utilizing many cycle, high peak power driving laser pulses, in combination with gating techniques [1], XUV pulse intensities up to 1014W/cm^2 have been reached in the spectral region 10-24eV. These pulses have been exploited in I) the temporal characterization of attosecond pulses [2] and II) the first proof of principle XUV-pump-XUV-probe experiments for the study of 1fs scale electron dynamics in atoms [3] as well as electronic, vibrational and ionization dynamics in molecules [4]. In this talk I will review these developments, perspectives towards their extension in complex systems such as large molecules, biomolecules and condensed matter samples and I will address opportunities that are opening up in these and related scientific topics by the up-coming Extreme Light Infrastructure – Attosecond Light Pulse Source (ELI-ALPS), one of the three pillars of the ESFRI roadmap European Research Infrastructure ELI.

Acknowledgements
This work is supported in part by the European Commission programs ATTOFEL, CRISP, Laserlab Europe, the European COST Actions MPI1203-SKO and CM1204 XLIC, and the Greek funding program NSRF.

References
[1] Tzallas P et al. (2007) *Nature Physics* 3: 846.
[2] Tzallas P et al. (2003) *Nature* 426: 267.
[3] Tzallas P et al. (2011) *Nature Physics* 7: 781.
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The Quantum Design of Photosynthesis

R. VAN GRONDELLE

VU University, Amsterdam, The Netherlands

Photosynthesis has found an ultrafast and highly efficient way of converting the energy of the sun into electrochemical energy. The solar energy is collected by Light-Harvesting complexes (LHC) and then transferred to the Reaction Center (RC) where the excitation energy is converted into a charge separated state with almost 100% efficiency. That separation of charges creates an electrochemical gradient across the photosynthetic membrane which ultimately powers the photosynthetic organism. The understanding of the molecular mechanisms of light harvesting and charge separation will provide a template for the design of efficient artificial solar energy conversion systems.

Upon excitation of the photosynthetic system the energy is delocalized over several cofactors creating collective excited states (excitons) that provide efficient and ultrafast paths energy transfer using the principles of quantum mechanics. In the reaction center the excitons become mixed with charge transfer (CT) character (exciton-CT states), which provide ultrafast channels for charge transfer. However, both the LHC and the RC have to cope with a counter effect: disorder. The slow protein motions (static disorder) produce slightly different conformations which, in turn, modulate the energy of the exciton-CT states. In this scenario, in some of the LHC/RC complexes within the sample ensemble the energy could be trapped in some unproductive states leading to unacceptable energy losses.

Here I will show that LHCs and RCs have found a unique solution for overcoming this barrier: they use the principles of quantum mechanics to probe many possible pathways at the same time and to select the most efficient one that fits their realization of the disorder. They use electronic coherence for ultrafast energy and electron transfer and have selected specific vibrations to sustain those coherences. In this way photosynthetic energy transfer and charge separation have achieved their amazing efficiency. At the same time these same interactions are used to photoprotect the system against unwanted byproducts of light harvesting and charge separation at high light intensities.

How to investigate the structure and function of light harvesting complexes

R. J. COGDELL

Institute of Molecular, Cell and Systems Biology, University of Glasgow, Glasgow, UK

This lecture will illustrate with examples from the purple photosynthetic bacterial light harvesting system, the methods with which the structure and function of these pigment protein complexes can be investigated. The topics covered will include x-ray crystallography, basic isolation and purification of light harvesting complexes and the use of ultra fast time resolved spectroscopies to investigate the energy transfer properties in real time.

Acknowledgement
RJC thanks the BBSRC and the US DOE for financial support.

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[1] Cogdell RJ, Gall A, Kohler J (2006) *Quarterly Reviews of Biophysics* 39: 227-324.

Light harvesting in the green alga *Chlamydomonas reinhardtii*

R. Croce

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The outer light-harvesting system of the model green alga *Chlamydomonas reinhardtii* is composed of 20 Lhc gene products, nine belonging to the antenna of Photosystem I (PSI), and eleven primarily to that of Photosystem II (PSII). In addition, light harvesting in PSI and PSII is regulated by the process of state transitions that in response to changes in light quality/quantity balances the excitation between the two photosystems by shuttling light-harvesting complexes (Lhc) between them. This process, which in plants involves 15% of the LHCII, was proposed to involve 80% of the Lhcb complexes in this alga. Finally, it was shown that *C. reinhardtii* is also capable of non-photochemical quenching, but only upon acclimation to high light which triggers the expression of LhcSR.

I will present the recent data that we have obtained on the characterization of the photosynthetic membranes of this algae. From the integration of biochemical, structural and functional measurements a complete picture of light harvesting and its regulation in this alga starts to emerge. We show that both PSI and PSII supercomplexes are far larger than the complexes of higher plants [1,2], but despite this the overall trapping efficiency is very similar. State transitions seem to work differenty than in plants, with most of the LHCII antenna becoming functionally disconnected from PSII but only part of it connects to PSI [3]. The largest PSI-LHCII complex, contains two LHCII trimers and one monomer in addition to the nine Lhcas [4]. Finally, *in vitro* and *in vivo* studies of non-photochemical quenchnig support the role of LhcSR as pH sensor in the membranes [5] and indicate the presence of two different quenching mechanisms.

Acknowledgement
the work presented in supported by the European Research Council (ERC) and the Netherlands Organization for Scientific Research (NWO).

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[1] Drop B, Webber-Birungi M, Fusetti F, Kouřil R, Redding KE, Boekema EJ and Croce R (2011) *J. Biol. Chem.* 286: 44878-44887.
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[5] Liguori N, Roy LM, Opacic M, Durand G and Croce R (2013) *J. Am. Chem. Soc.* 135: 18339-18342.

New uses of an old method, the x-ray diffraction

G. FAIGEL

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X-ray diffraction is about 100 years old. In the past century it was highly refined and today it is the most widely used method for structure determination. In this talk the most important problems of x-ray structure determination - the missing phase [1-6], the radiation damage [7,8], and the non-availability of single crystals - are outlined, and some solutions based on recent technical developments of x-ray sources [9-12], detectors and computer capabilities are given. Further, the possibility of following structural changes in short time scales is discussed.

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[11] Planned Research Activity of ELI-ALPS – part of the Feasibility Study (30 October, 2012)
[12] ELI Preparatory Phase - White Book

Imaging Experiments with Free Electron Lasers

J. HAJDU

Laboratory of Molecular Biophysics, Uppsala University, Sweden

Theory predicts that with an ultra-short and extremely bright coherent X-ray pulse, a single diffraction pattern may be recorded from a large macromolecule, a virus, or a cell before the sample explodes and turns into a plasma. The over-sampled diffraction pattern permits phase retrieval and hence structure determination. X-ray lasers capable to deliver ultra bright and very short X-ray pulses for such experiments have recently started operations. Free-electron lasers are the most brilliant sources of X-rays to date, exceeding the peak brilliance of conventional synchrotrons by a factor of 10 billion, and improving. In the duration of a single flash, the beam focused to a micron-sized spot has the same power density as all the sunlight hitting the Earth, focused to a millimetre square. The interaction of an intense X-ray pulse with matter is profoundly different from that of an optical pulse. A necessary goal of research is to explore photon-material interactions in strong X-ray fields. Our aim in biology is to step beyond conventional damage limits and develop the science and technology required to enable high-resolution imaging of biological objects. The talk will summarise imaging results from the Linac Coherent Light Source.

Watching Photosystem II at Work Using fs X-ray Pulses

J. KERN[1,2], R. TRAN[1], J. HATTNE[1], S. KOROIDOV[3], J. HELLMICH[4], R. ALONSO-MORI[2], N. K. SAUTER[1], U. BERGMANN[2], J. MESSINGER[3], A. ZOUNI[4], J. YANO[1], V. K. YACHANDRA[1*]

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Photosystem II (PSII) catalyzes the light driven oxidation of water to dioxygen and protons. The catalytic site of this multisubunit membrane protein complex is a Mn_4CaO_5 cluster, located at the lumenal side of the complex. Upon light exitation it cycles through a series of states (S0 to S4) with S1 being the dark stable state. Despite a recent 1.9 A structure [1] many details of the reaction mechanism are still unknown, especially, as the Mn-cluster is highly susceptible to radiation damage [2]. X-ray spectroscopic methods (XANES, EXAFS and Mn Kβ X-ray emission (XES)) are valuable complementary tools to diffraction studies to investigate the oxidation state and electronic structure of the metal cluster. They also allow for direct monitoring of radiation damage to the catalytic site [2,3]. On the other hand X-ray diffraction data yield orientation information that can be used to evaluate polarized X-ray spectroscopy on single crystals [4]. The ultrafast (fs) pulses of X-ray free electron lasers (XFELs) allow performing X-ray measurements on radiation sensitive samples at room temperature in a shot-by-shot measurement before manifestation of X-ray induced changes of the sample, using the "probe-before-destroy" concept [5]. We conducted combined XES/XRD studies at the first operational hard X-ray XFEL, the LCLS at Stanford. Here we collected room temperature XRD from micro crystals of PSII in the dark and some illuminated states using sub-50fs X-ray pulses [6-8]. Simultaneously XES was collected to monitor the intactness of the catalytic site [7] and follow changes of the oxidation state of the Mn cluster [8].

Acknowledgement
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The best of both worlds: SMALPs for solubilised photosynthetic membrane proteins with native-like properties

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The use of detergents to solubilise membrane proteins and allow their study *in vitro* is often essential for their characterisation and/or utilisation. However, finding the optimal detergent for stability and function can be a challenging and laborious task. Even with the appropriate detergent compromises are often made in the stability and function of purified proteins. To overcome these issues many researchers have turned to membrane-like systems such as liposomes or nanodiscs stabilised by a protein scaffold. Whilst these methods improve the properties of the protein, they are time consuming and still require removal of the protein from the native environment by detergent solubilisation.

Recent advancements in the use of styrene maleic anhydride (SMA) polymers have shown that proteins can be extracted into nanodisc-like structures directly from the membrane, which are termed SMA-lipid-particles (SMALPs) [1]. Using the photosynthetic reaction centre (RC) from purple bacterium *Rhodobacter sphaeroides* as a model system, we show that RC-SMALPs retain the functional characteristics of the protein in its native membrane environment [1]. However, unlike in native membranes the protein can be purified to provide the flexibility of a detergent-solubilised system. In our studies we find that the native lipid composition of the membrane has been retained within the RC-SMALPs. This yields reaction centres with biophysical characteristics remarkably similar to the native membrane whilst preparation with detergents shows significant differences. Simultaneously SMALPs do not have issues of light scattering and lack of control over the presence of additional components such as other interacting proteins associated with the native membrane. This yields a system in which we have the advantages of the protective native membrane environment and solubilised protein, giving the best of both worlds.

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Responsive matrices for solar to fuel conversion: trading time for efficiency

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In natural photosynthesis, the reaction time imposed by thermodynamics is exploited for kinetic control and efficient conversion of solar energy. The reaction coordinate is made essentially barrier-less by allowing time for tunneling, while coherent motion is exploited to handle energy differences for rapid transfer and conversion of excitons into charge separated states. In bacterial photosynthetic reaction centers, excitation of the special pair (P) couples to specific low frequency intradimer and protein modes to provide an efficient channel for vibrationally assisted mixing of charge transfer states into excitons. This process has been described with a modified Redfield Hamiltonian that includes four energy levels and two collective modes. With solid state NMR and modeling we have been able to map this minimal Redfield model on a reaction coordinate for the special pair and its protein environment. Photo-CIDNP enhanced solid state Magic Angle Spinning (MAS) NMR chemical shift and homonuclear dipolar correlation data for the electronic ground state reveal how the special pair is tuned for electron transfer by packing effects that give rise to protein-induced misfits on chlorophyll cofactors [1]. The axial histidines produce $P_L^{\delta-}P_M^{\varepsilon-}$ charge transfer character. This configuration is unstable and oscillates between the two halves of P due to intradimer vibrations, which can be detected with solid state NMR spin diffusion methods. Every time the pair is in a favourable charge transfer configuration, charge separation can proceed efficiently, and electrons can tunnel from the donor into the redox chain of the active branch of the reaction center. The 3-D specific motions involve classically coherent charge transfer intermediate formation between the two halves of P, HisM202 rotation, proton displacement coupled to this motion involving water and proton displacement upon oxidation of P involving HisL168 and the 3^1-acetyl of P_L [1]. Overall the reaction is slowed down by the oscillatory behaviour of P, but it can proceed with little energy loss because of temporal lowering of the transition state by dynamic structural changes that lower the energy levels and can confine charge for dissipative, proton-assisted tunnelling. This is different from the Marcus transfer mechanism that requires a match between the free energy difference and the reorganization energy in an incoherent process, and it is also different from the depletion region or "band bending" that drives charge separation in majority carrier solar cell and PEC systems. The time-dependent alternation between quantum delocalization and classical confinement makes effective use of non-stationary states that evolve in time, and get "dressed" with selected vibrations on a very short time scale, a few tens of femtoseconds. The protein can control the vibration dressing, and by adapting the structure the selected modes that can contribute to a smooth reaction coordinate will be the modes that are enhanced. This will result in semi-classical evolution along the reaction coordinate with an effective particle mass of ~ 100 electron masses, which can appear as an effective driver of natural and artificial photosynthesis.

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Building principles of photosynthetic light-harvesting antennae: Theory meets experiment

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In order to bridge the gap between the crystal structures of photosynthetic light-harvesting proteins and optical experiments probing their function, two essential problems need to be solved [1]. On one hand, theories of optical spectra and excitation energy transfer have to be developed that take into account the pigment-pigment (excitonic) and the pigment-protein (exciton-vibrational) coupling on an equal footing. On the other hand, the parameters entering these theories need to be calculated from the structural data. I will give a summary of recent approaches to solve the above problems and present applications to different antenna proteins revealing different strategies developed in these systems for efficient light-harvesting. Latest theoretical developments concern the inclusion of non-secular terms in the theory of linear absorbance and circular dichroism spectra of pigment-protein complexes [2], and the calculation of the spectral density of the pigment-protein coupling by a combined qunatum chemical/electrostatics/normal mode analysis (NMA) approach [3]. Non-secular terms are found to redistribute oscillator strength between exciton transitions. The magnitude of this effect is in the 10 percent range of the overall signal. Application of the theory to the water-soluble chlorophyll binding protein (WSCP) reconstituted with chlorophyll (Chl) *a* and Chl *b* reveals slight differences in the transition dipole geometries between Chl *a* and Chl *b* homodimers. From the NMA of the spectral density of the Fenna-Matthews-Olson protein, information is obtained about how the protein dissipates the excess energy of excitons. The fluctuation of transition energies, induced by the protein dynamics, is found more than one order of magnitude stronger than the fluctuation of excitonic couplings and the correlation in transition energy fluctuations has practically no influence on exciton relaxation, because the relevant frequencies are different.

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21st century challenges at the interface of biology and computer science

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Biology and computer science are two fields that witnessed an enormous expansion in the last decades. Bioinformatics lies at the interface of these two fields and benefits from their synergy.

Progress in genomes sequencing techniques and in available computational power makes possible to face new challenges in biology and medicine. With the structural bioinformatics tools now available it is becoming reality the possibility to obtain structural data on a protein starting just from its gene sequence [1], to study *in silico* the binding of substrates and drugs to proteins of biomedical interest [2] and to design novel proteins which carry out desired functions of industrial relevance [1]. Further, structural bioinformatics techniques have a great potential especially in the case of membrane proteins whose structure is often difficult to solve with experimental methods [3].

The lecture will review these recent advances in the field of structural bioinformatics with prominent examples taken from the literature and from the research activity carried out in the Bioinformatics lab of the Roma Tre University.

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Molecular Basis of Photoprotection in Natural and Artificial Photosynthesis

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Through their light-harvesting antenna, plants frequently absorb more solar energy than they can use in photosynthesis. This excess energy has the potential to cause cell damage, such as pigment bleaching and protein inactivation. To minimise photodamage, a number of protection mechanisms exist, which we have characterized at a molecular/functional level. In particular, the photosynthetic process promotes with a low, but significant yield, chlorophyll triplet states, which are potentially able to sensitize singlet oxygen. In oxygenic photosynthetic organisms, chlorophyll triplet states are extremely efficiently quenched by carotenoid molecules.

We recently studied, triplet-triplet transfers between carotenoid and chlorophyll molecules, and more generally between carotenoid molecule and pophyrins to understand the molecular origin of the observed ultrafast triplet-triplet transfer in light harvesting complexes of plants and algae. Our results suggest that the efficiency of this transfer is liknked with a partial delocalization of the triplet state onto the donor and acceptor molecules.

Femtosecond stimulated Raman spectroscopy in 1D and 2D – basics and beyond

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It is now more than 50 years since the discovery of stimulated Raman phenomena and 15 years since establishing the femtosecond stimulated Raman (FSRS) techniques [1]. Nevertheless stimulated Raman measurements as an analytical probe are far from being a widespread and universal tool and very few successful studies of complex systems such as proteins have been reported so far. Among the major problems is the unresolved issue of a proper rejection of all parasitic signals. We are employing pulse shaping techniques to achieve a complex modulation of Raman signal in the frequency domain in order to make signal filtration more robust and fully universal [2]. Additionally shaping of the temporal profile of the Raman pulse can in itself reduce artifacts associated with the time resolved FSRS measurement. Such approaches are crucial in order to apply FSRS on proteins and other soft matter samples. By linking two fs lasers through a a shared fs-oscillator we can perform time resolved Raman studies, where time evolution from fs to seconds is investigated within a single experiment.

In addition to the classical 5th order time resolved FSRS experiment (fs pump-delay-FSRS probe) we are investigating 5th order and 8th order 2D Raman experiments targeted on revealing mutual coupling among the vibrational modes of studied systems. Such 2D spectra should be very explicit probe to any structural changes of molecules. Although 2D Raman experiments face severe difficulties in comparison to 2D mid-IR spectroscopy, if successful, such techniques are expected to be much more powerful than their mid-IR counterparts due to tunable selectivity through resonance enhancement, unlimited spectral window, and possibility to harness very mature vis-near-IR photonics. In addition to this 2D Raman experiment is potentially much cheaper to implement and run. In contrast to previous attempts [3] we are investigating a frequency domain techniques, with expectation to have more space to reject notorious cascading signals that shed a considerable skepticism [4] on time domain 2D Raman techniques.

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Davydov States for Energy Transfer Dynamics in Light Harvesting Systems

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The Davydov D_1 ansatz, which assigns individual bosonic trajectories to each spin state, is an efficient, yet extremely accurate trial state for time-dependent variation of the sub-Ohmic spin-boson model [1]. Variants of the ansatz have been successfully applied to simulate energy transfer dynamics in large light-harvesting systems including thousands of pigments coupled to a continuum spectrum of phonons [2,3]. Extensions to multiple D_1 ansatze produce nearly exact ground states of model Hamiltonians. In addition, a surface hopping algorithm is developed employing the Davydov D_1 ansatz to study the population dynamics with a sub-Ohmic bosonic bath. The algorithm takes into account both coherent and incoherent dynamics of the population evolution in a unified manner, and compared with semiclassical surface hopping algorithms, hopping rates calculated in this work follow more closely the Marcus formula [4].

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Terahertz Measurement Technology in Photobiophysics and at ELI-ALPS

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Terahertz (THz) science and technology is a relatively new, but fast developing field [1,2]. THz pulse generation and detection methods using ultrashort laser pulses especially advanced this field [3]. Such methods enable to measure the transient electric field (rather than the intensity). In THz spectroscopy [4,5] this enables the direct determination of both the amplitude and phase of each spectral component, that is the determination of both the real and imaginary parts of the complex permittivity. Time domain THz spectroscopy is sensitive to the conformation of biomolecules [6], and very sensitive to the state of the water network [7]. THz pulses with μJ level energy are generated routinely [3], enabling THz pump–THz probe measurements for dynamical investigations [8]. From the point of view of photobiophysics it is especially important that by using THz techniques it is also possible to measure the temporal shape of THz radiation emitted by ordered materials. For example the fundamental primary charge translocation during the functional energy conversion process of bacteriorhodopsin was revealed in this way [9].

Besides a short overview of ultrashort THz pulse generation and detection methods and mentioning a few application examples, the planned THz facility of ELI-ALPS will be introduced in the talk.

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THz vibrational study of biomolecules

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Terahertz spectroscopy is a powerful technique to provide information on low energy molecular state transitions, intermolecular bonding and intramolecular vibrations. The received *time domain terahertz spectroscopy* (TDTS) data is converted by Fast Fourier Transformation (FFT) to transmittance spectrum then to absorbance spectrum on interest.

The structure and as a consequence the vibrations of proteins depend on the physico-chemical properties of the environment and the molecular interactions that they are involved. The amount of collective modes increasing by molecule size and complexity hinder characteristic spectroscopic responses to THz radiation, therefore spectroscopic comparison of structural changes requires specific techniques. We investigated biologically relevant molecules to study their spectral response to THz radiation and found matching spectral features with predicted ones by quantum chemical molecule modelling (DFT). Characteristic absorbance peaks were determined for amino acids, peptides and specific proteins of our interest.

This fundamental research was carried out to discover the relationship between the complex spectrum of polypeptide chains and its building elements; the amino acids.

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Light-Harvesting, antenna quenching, and ultrafast charge separation in reaction centers. A unified view.

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Comparing pigment arrangements in the many available structures of photosynthetic antenna complexes with those appearing in reaction centers, one notices that very similar arrangements of the same pigments have been chosen to function either as efficient light harvesters or as centers of ultrafast charge separation. It thus becomes obvious that the detailed pigment arrangement is not the main factor controlling excited state processes. Rather it is the surrounding protein that actually decides about the specific functioning in a particular case, i.e. whether a pigment cluster functions as an antenna or as a reaction center (charge separator). Thus the protein – or more generally the "environment" - takes the role of a "smart responsive matrix". At present we do not understand well the governing factors at a molecular level. However the design of efficient artificial antenna and charge separation units – with or without proteins – requires a deeper understanding and control of these environmental effects, i.e. the properties of the "responsive matrix".

Recent studies on non-photochemical quenching (NPQ) in antenna complexes have turned out to provide fundamental insights into these environmental effects controlling the fate of the excited state energy. A common denominator of most NPQ quenching processes is the ultrafast formation of a chlorophyll-chlorophyll charge transfer state, by a mechanism that in many details operates in the same way as the "normal" processes of ultrafast photosynthetic charge separation occurring in reaction centers. Astounding and unexpected parallels between those - at first glance unrelated - processes of NPQ and of reaction center function have been found.

The talk will discuss the general common principles governing the role of the surrounding protein in switching excitonically coupled pigment arrangements between light-harvesting, non-photochemical quenching, and energy-storing by ultrafast charge separation in reaction centers.

Acknowledgement
I acknowledge all my outstanding coworkers over several decades who have with their work helped to shape this unified view on fundamental aspects of several seemingly unrelated photosynthetic processes. This work has been supported over the years by many funding organizations. Most recently I acknowledge financial support by the project DFG HO-924/3-1of the Deutsche Forschungsgemeinschaft, the Eurocores EUROSOLARFUELS programme, and the EU Training and Research Network "Harvest" of the European Union.

Photosynthetic complexes studied with picosecond spectroscopy: from dead crystals to living cells.

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Light harvesting and subsequent electron transfer occur predominantly on a time scale of picoseconds to nanoseconds [1,2]. Fluorescence (micro)spectroscopy can be used to study these first events in photosynthesis both *in vitro* and *in vivo*. One of the aims of this type of research is to correlate the obtained results with the crystal structures of the pigment-protein complexes involved and to understand the underlying mechanisms that make these complexes function. On the other hand we would like to understand how these complexes work together *in vivo*, both in normal and stress conditions. We have compared the fluorescence kinetics of photosystems I [3] and II [4] in crystals and in detergent and demonstrate that their properties are very similar, which is important when modelling the spectroscopic properties of these complexes using the available crystal structures. On the other hand it appears that the fluorescence kinetics of light-harvesting complex II (LHCII) differs for crystallized and solubilized LHCII and even depends on the crystal form and the way of solubilizing [5,6]. This is possibly related to its biological role as a light-harvesting complex that in high-light conditions can switch into a quenched form [7]. The above results will be presented together with recent results on non-photochemical quenching and state transitions in different organisms [8-11].

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"Biological Variability" in the Research of Photosynthetic Light Harvesting

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Photosynthesis nourishes nearly all the life on the Earth either directly or indirectly. It begins with the absorption of solar energy by a collection of protein complexes with highly organized and densely packed pigment chromophores, generally known as the light harvesting complexes. The captured by the light harvesting complexes solar energy is eventually transferred to the reaction center, where the photochemical charge separation takes place. In most of the purple bacteria, the photosynthetic membrane is composed of two types of closely packed arrays of light harvesting complexes: the core light harvesting complex 1 (LH1) and the peripheral/distal light harvesting complex 2 (LH2). While the architecture of LH2 is relatively stable, the LH1 design varies strongly from species to species. The LH1 forms a closed ring around the reaction center in *Rhodospirillum rubrum*, whereas in *Rhodopseudomonas viridis* and in *Rhodospirillum photometricum,* it is a closed ellipse around it. Some species of photosynthetic bacteria like *Rhodobacter sphaeroides* hold an additional polypeptide, PufX, which creates a gap in the LH1 ring around the reaction center. The presence of the PufX polypeptide in wild type *Rhodobacter sphaeroides* leads most of the core proteins to associate in a way to form S-shaped dimeric core complexes, while a few percentages of them still remain monomeric. The core complexes are interspersed and interconnected by the distal LH2 complexes. The unified structural organization of light harvesting complexes and reaction centers also remarkably depends on the light intensity at which the bacterial cells were grown.

Here, we report on the thorough experimental investigations about the influence of the growth conditions and the structural build-up (monomeric or dimeric) of core complexes on the energy transfer rate and the efficiency of collecting energy in the intacytoplasmic membranes of *Rhodobacter sphaeroides*. The study, which involved various steady-state and picosecond time-resolved spectroscopic techniques, clearly revealed the significant changes in the rates of delivery of the excitation energy to the reaction centers and the quantum efficiency of charge separation in different membrane cultures. Among other issues, the observed variations contribute into our understanding of the many differences in the literature data, often indistinctly related to "biological variability". Part of this study was recently published [1].

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Carotenoids in Regulation of Energy Flow through Photosynthetic Antenna – Light Harvesting vs. Photoprotection

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Carotenoids are key constituents of photosynthetic light-harvesting systems in which they fulfil various functions. The diversity of carotenoid functions is directly related to their unique spectroscopic properties resulting from a structure of carotenoid molecule. The central pattern repeated in all carotenoids is a backbone consisting of alternating single and double carbon bonds that forms a conjugated π-electron system responsible for most of the spectroscopic properties of carotenoids. The bright yellow-orange color of carotenoids is caused by a strong transition to the excited state called S_2. Due to symmetry reasons, the transition to the lowest excited state (S_1) is forbidden, thereby restricting observable absorption or fluorescence. After excitation, the S_2 population relax to the S_1 state within a few hundreds femtoseconds, while the lifetime of the S_1 state varies between 1-300 ps depending on conjugation length of the carotenoid [1]. This standard picture has been significantly modified during the past decade by introducing other dark states, which may be located between (or in close vicinity of) the S_1 and S_2 states, making the excited state dynamics vastly complicated [1,2].

In photosynthetic systems, carotenoids are crucial constituents of light-harvesting proteins, in which they carry out two rather orthogonal functions. First, there is a conclusive evidence that carotenoids are capable to transfer captured energy to (bacterio)chlorophylls with efficiency ranging from 0-100% [3]. Second, carotenoids serve also as photoprotective agents protecting photosynthetic proteins against excess light. In many systems, carotenoids are capable of carrying out these two functions simultaneously, which makes them rather unique molecules. It now becomes obvious that these specific properties are often related to a particular configuration of a carotenoid molecule which is one of the key features allowing carotenoids to fulfil a broad range of functions. The role of changes in carotenoid local structure induced by protein binding sites in tuning the light-harvesting and/or photoprotective functions of carotenoids will be demonstrated by a few examples of light-harvesting and photoprotective strategies in photosynthetic microorganisms. Potential use of ELI-Beamlines and/or ELI-ALPS facilities for future studies of carotenoids will be discussed.

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Distinctive Character of Electronic and Vibrational Coherences in Photosynthetic Pigment-Protein Complexes

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Quantum behaviour of the excitation dynamics is widely discussed by analyzing the two-dimensional (2D) electronic coherent spectra of various pigment-protein complexes [1]. Depending on the coupling between electronic and vibrational states, oscillating signals in the 2D spectroscopy could evidently be attributed to purely electronic, purely vibrational or mixed origin [2]. Even in the "mixed" molecular systems two types of coherent beats reflecting either electronic or vibrational character can be distinguished by analyzing oscillation Fourier maps, constructed from evolution of the 2D spectra [3,4]. The amplitude of the electronic-character beatings is heavily affected by the inhomogeneous disorder and consequently electronic coherences are quickly dephased. Beatings with the vibrational-character depend weakly on the electronic disorder, assuring their long-time survival [4,5]. We show that modeling of 2D spectroscopy signals of vibronically-coupled system provides direct information on the origin of the coherent beatings. 2D spectra of the PSII reaction center [6,7], and Fucoxanthyn-Chlorophyll proteins [8] are analyzed by using this type of model considerations.

Acknowledgement
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Ultrafast multi-dimensional electronic spectroscopy and its applications to the study of LHCII

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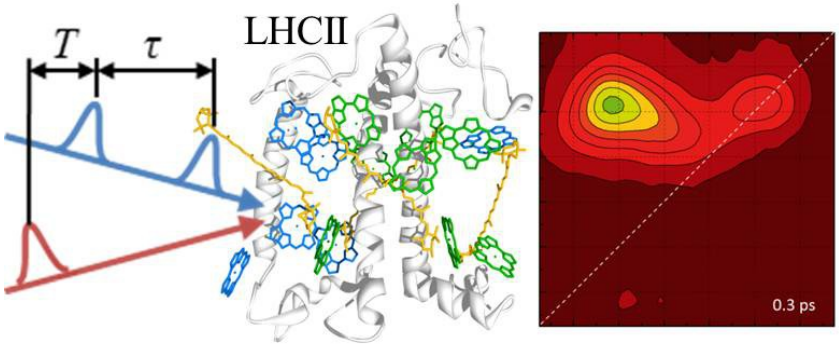
Recently, there has been much interest in the application of ultrafast multi-dimensional electronic spectroscopy to the study of Light Harvesting Complexes [1]. We will review the basic principles of multi-dimensional electronic spectroscopy.



We report on our application of ultrafast two-dimensional (2D) and three-dimensional (3D) spectroscopies [2,3] to the study of the excitonic energy transfer (EET) processes of LHC II light harvesting complexes [4]. The EET process in light harvesting complexes is typically complex and proceeds in a multistep fashion. In 2DES, the spectra are presented in two frequency dimensions: one excitation frequency and one of the emission frequency. The crosspeaks on the 2D spectra correlates the donor exciton to the acceptor exciton. As this is a two point correlation, multistep processes can only be indirectly observed. In 3DES, with an additional frequency axis, three-step processes can be directly observed. A crosspeak on a 3D spectrum at (ω_a , ω_b , ω_c) will denote exciton *a* transferring energy to exciton *c*, via an intermediate exciton *b*. We have performed 3DES on LHCII trimers, and *directly* observed for the first time multistep EET process.

We also present recent results in our studies of the ultrafast dynamics of energy transfer from Chl *b* to Chl *a* band in trimeric and aggregated LHCII are examined using two-dimensional (2D) electronic spectroscopy. Global fitting analysis of the 2D spectra revealed similarities and differences between the kinetic components of LHCII trimers and aggregates. The 2D decay-associated spectra resulting from global analysis resolved an intermediate midenergy state of which the decay pathway depends on the physical state of LHCII.

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Mapping energy transfer flow in fucoxanthin-chlorophyll protein complex

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Fucoxanthin–chlorophyll protein (FCP) is the key molecular complex performing the light-harvesting function in diatoms, which, being a major group of algae, are responsible for up to one quarter of the total primary production on Earth [1,2]. These photosynthetic organisms contain an unusually large amount of the carotenoid fucoxanthin, which absorbs the light in the blue–green spectral region and transfers the captured excitation energy to the FCP-bound chlorophylls. Due to the large number of fucoxanthins, the excitation energy transfer cascades in these complexes are particularly tangled.

Coherent two-dimensional (2D) electronic spectroscopy (ES) provides a wealth of information about the energy and charge transfer dynamics, exciton diffusion and relaxation in molecular systems. In the 2D ES the temporal and spectral resolutions are not related, providing a huge advantage over the pump-probe techniques [3]. Usually in the 2D ES, all the excitation pulses are of the same wavelength, or color. However, it may be hugely advantageous to tune them to different energies, thus providing a way to monitor energy transfer between energetically remote excited states [4].

In this work we present the two-color two-dimensional electronic spectroscopy experiments on FCP. Analysis of the data using modified decay associated spectra permits a complete mapping of the excitation frequency dependent energy transfer flow with a femtosecond time resolution. It is striking, how the protein organization is able to bind fucoxanthins and chlorophylls, maintaining dramatically different absorption properties, in such a way, that the spectral range for photon absorption by the whole protein is significantly enhanced and, at the same time, ultrafast and efficient excitation energy cascade from all these molecules is ensured.

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Metallosubstituted (Bacterio)chlorophylls - Ultrafast Molecular Devices for Studies of Photosynthetic Processes in Time and Space

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The central metal ions, especially if they are transition metals, can substantially change the photophysical features of (bacterio)chlorophylls [1,2]. We have recently shown that the central metal bonding in these complexes crucially depends on the symmetry of the ligand field created by the central binding pocket and the valence configuration of the metal center [3]. This is a fundamental difference between metalloporphyrins and (bacterio)chlorophylls. In particular, the central Mg^{2+} is bonded by strictly electrostatic forces while Zn^{2+} forms classical coordinative bonds and Ni^{2+} strong mixed coordinative-covalent bonds [4]. The latter type of bonding is especially interesting because it introduces novel photophysical properties to the complex, manifested e.g. in a drastic shortening of its excited state lifetime. Within a few tens of femtoseconds, the energy of the absorbed photons is converted into heat with a 100% efficiency. The Ni-substituted analog of (bacterio)chlorophyll is able replace the native pigments in photosynthetic pigment-protein complexes, which can be exploited in the investigations of the mechanisms of their action. For instance, it was used to estimate the range of migration of excitons in photosynthetic antennae. In addition, the effects of the Ni-substituted pigment on the antenna complex properties provided insights into spatial and structural relationships within the complex, such as the size of antenna functional units [5-7]. So far, thanks to the ease of the reconstitution and pigment exchange, this approach has been applied to study the primary antenna processes in photosynthetic bacterial LH1 complex. Now, our goal is to extend this experimental approach to major plant antenna LHCII in order to achieve a better understanding of intracomplex energy transfer and pigment-pigment couplings in this complex.

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Protein Dynamics as a Factor Influencing the Intraprotein Electron Transfer

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Electron transfer inside protein depends on many factors including the chemical nature of electron carriers embedded in the protein and their interactions with the neighborhood. The latter factor may be modulated by electrical charges inside the protein (related for e. g. to functional state of the protein and pH), temperature, pressure, nature of solvent surrounding the protein and likely other factors. Importantly, protein is not a static environment but a dynamic one with structural rearrangements occurring on a wide range of time scales from ultrafast (~picoseconds) to very slow (~seconds or slower). Therefore, it may be expected that the protein dynamics is an additional factor influencing the intraprotein electron transfer.

The protein dynamics may be either spontaneous or stimulated by changes in an electrostatic field inside the protein. These changes may be caused for e. g. by appearance of new charges inside the protein. A convenient system for studying this effect are photosynthetic reaction centers, proteins specialized in electron transfer. In terms of studying the influence of protein dynamics on intraprotein electron transfer, situation is a bit complicated in such a system but manageable: on one hand, electron transfer induces protein dielectric response, and on the other hand, this protein response influences the electron transfer.

During the lecture, it will be demonstrated how to extract the rates of protein dynamics from the transient absorption measurements of $P^+H_A^- \rightarrow PH_A$ charge recombination electron transfer reaction performed on reaction centers from *Rhodobacter sphaeroides* purple bacteria (P and H_A are the primary electron donor and acceptor, respectively). By using different single amino acids mutants and performing experiments at a range of temperatures it was possible to conclude on the influence of these factors on protein dynamics [1-3].

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Biosolar cells: natural and artificial assemblies of light energy transducing protein complexes

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The harvesting of solar energy in photosynthesis is dependent upon an interconnected macromolecular network of membrane associated chlorophyll-protein complexes. In the past decade my workgroup and others have elucidated the structure and functioning of these networks to great detail. Here I will briefly discuss our results of high resolution AFM imaging of native membranes and the models derived from light spectroscopy [1,2]. In the second part I will discuss our recent efforts in applying and mimicking the natural assemblies in hybrid biosolar cells, photosynthesis based electrodes as components for sensors [3], photovoltaics [4] and, possibly, photofuels. If time allows, I end with our recently designed algae powered robot which showcases the possibilities (http://www.raoulfrese.nl/the-symbiotic-machine/).

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Pigment Interactions in Light-Harvesting Complex II in Different Molecular Environments

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Extraction of the plant light-harvesting complex II (LHCII) from the native thylakoid membrane or from aggregates by the use of surfactants brings about significant changes in the excitonic circular dichroism (CD) spectrum and fluorescence quantum yield. To elucidate the cause of these changes, e.g. trimer-trimer contacts or surfactant-induced structural perturbations, we compared the CD spectra and fluorescence kinetics of LHCII aggregates, artificial LHCII-lipid membranes, and LHCII solubilized in different detergents or trapped in polymer gel. By this means we were able to identify CD spectral changes specific to LHCII-LHCII interactions - at (-)437 nm and (+)484 nm - and changes specific to the interaction with the detergent n-dodecyl-β-maltoside (β-DM) or membrane lipids - at (+)447 nm and (-)494 nm. The latter change was attributed to the conformation of the LHCII-bound carotenoid neoxanthin, by analyzing the CD spectra of neoxanthin-deficient plant thylakoid membranes. All aggregation-specific features were identified in native thylakoid membranes from lincomycin-treated plants which contain only light-harvesting complexes but no photosystem core proteins. Neither the aggregation-specific CD bands, nor the surfactant-specific bands were positively associated with the onset of fluorescence quenching, which could be triggered without invoking such spectral changes. Significant quenching was not active in LHCII membranes, while a high degree of energetic connectivity, dependent on the lipid:protein ratio, allows for efficient light harvesting.

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Efficient Energy harvesting with nanostructured hybrid systems containing photosynthetic pigment-protein-complexes

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A comparative study of the EET in the phycobiliprotein (PBP) antenna of the Chl *d*-containing cyanobacterium *Acaryochloris marina* and decomposed antenna subunits at room temperature (RT) using time resolved absorption and fluorescence spectroscopy has revealed the EET transfer mechanisms and times for the PBP antenna in *A.marina*. The kinetics that were obtained show that EET processes over the PBP-antenna in *A. marina* occur at RT with time constants of 3 ps and 14 ps followed by a subsequent transfer to the Chl *d* containing reaction center with a time constant of 30-40 ps [1]. It was possible to form hybrid systems in aqueous buffer solution by self-assembly of different CdSe quantum dots (QDs) surrounded by a ZnS shell and functionalized by covering the surface with anionic and cationic groups together with the rod-shaped PBP antenna from *A. marina*. EET from QDs to PBP rods was found to take place with varying and highly temperature-dependent efficiencies of up to 90% with a corresponding fluorescence rise in the acceptor material. The used CdSeQDs exhibit fluorescence emission at 530 nm and transfer the excitation energy to the PBP antenna within 140 ps – 240 ps at room temperature. The luminescence of the PBP antenna occurs with maxima at 650 nm (PC) and 665 nm (APC). The mode of binding and coupling change with the size of QDs and with temperature [2].

At room temperature an efficient EET from the QDs to the PBP antenna of *A.marina* is found with a heterogenic efficiency distribution including strongly coupled QDs with > 90 % EET efficiency and an overall average EET efficiency of 78 % [2]. In addition marked configuration changes must occur around 0°C. This transition leads to an interruption of the EET at low temperatures that can not be explained by simply spectral narrowing at lower temperatures [3].

For large QDs the semiconductor material can function as EET acceptor. For such applications sensitive molecular couplings strongly dependent on the structural organisation of such complexes play an important role [3]. The Förster overlap integral turned out not be be the main determining factor for efficient EET transfer as proposed from theory [3]. Interestingly the electrostatic coupling leads to large clusters containing complex structured hybrid materials with QDs and PBP antenna complexes. Such materials might be of highest relevance to overcome the problems of low absorbance in monolayers with an additional efficient EET between biological and semiconducting material. The localized injection of electron-hole pairs into the semiconducting material is suitable to reduce the recombination rate for more than an order of magnitude imitating the situation in a plant light harvesting system including reaction center. TiO_2 tubes forming arrays of rods spaced by several tens of nm and several hundreds of nm in height can collect biological antenna systems and hybrid systems from RCs and QDs. The charge is localized near the contact of the TiO_2 rods to ITO. As cooling leads to a decoupling of the PBP antenna complexes and the QDs low temperature "shocks" can be used to refresh the PBP structures by cooling, subsequent washing and addition of fresh biological structures that re-selfassemble with the remaining semiconductor structure.

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Light Harvesting in a Fluctuating Antenna

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Photosystem II (PSII) is a huge pigment–protein supercomplex serving as a starting point for oxygenic photosynthesis and ensuring both very efficient light harvesting and subsequent excitation energy transfer towards the reaction center (RC). Time-resolved spectroscopic measurements of PSII usually reveal complex multi-exponential fluorescence decay kinetics. Since such a non-exponential behaviour persists independently of the sample preparation and the actual antenna size of the studied PSII [1], it has been for decades ascribed to be caused by the reversible charge separation taking place in the RC. However, in this description the protein dynamics is not taken into consideration. Meanwhile, the intrinsic dynamic disorder of the light-harvesting proteins along with their fluctuating dislocations within the antenna [2] inevitably result in varying connectivity between pigment–protein complexes and therefore can also lead to non-exponential excitation decay kinetics. To account for this effect, we propose a simple conceptual model [3] that describes excitation diffusion in a continuous medium of fractional dimensionality and deals with possible variations of the excitation transfer rates. Recently observed fluorescence kinetics of PSII of different sizes [1] are perfectly reproduced (Fig. 1) by using only two adjustable parameters instead of the many decay times and amplitudes required in standard analysis procedures; no charge recombination in the RC is required. The proposed model also straightforwardly solves various contradictions currently existing in the literature and can provide valuable information about the structural and functional organization of the photosynthetic antenna, as demonstrated by applying it to the fluorescence decay kinetics in the thylakoid membranes.

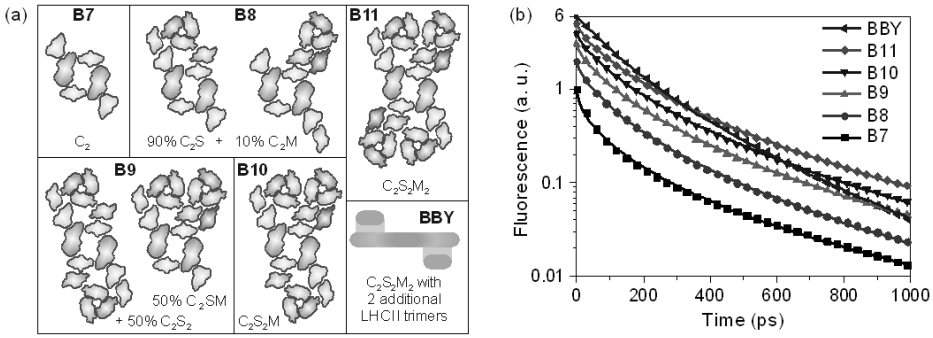

Fig. 1. (a) Schematic structures of variously sized PSII supercomplexes. (b) Experimental (symbols) and simulated (lines) multi-exponential fluorescence decay kinetics in various PSII supercomplexes. For visual clarity, fluorescence kinetics in B8–BBY supercomplexes were multiplied by integer numbers 2–6, respectively.

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Protein-based high speed all-optical logic

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Photonics is considered to be a complementary of conventional electronics in future informational technology. Having the theories well-developed for optical data processing, the basic problem is to find proper nonlinear optical (NLO) materials that are able to actively control optical circuits.

Suitable NLO materials with high stability and sensitivity are being intensively researched [1]. Besides organic and inorganic crystals, the chromoprotein bacteriorhodopsin (bR) has generated the most interest for optoelectronic applications [2,3]. bR, isolated from the outer cell membrane of the bacterium *Halobacterium salinarum*, is the simplest known ion pump, and one of the best-characterized membrane proteins. Upon illumination it transports protons across the cell membrane, meanwhile the molecule changes its optical absorption, refractive index and charge distribution. These properties can be used separately or simultaneously in opto-electronic devices [4-7]. The operation of bR-based ultra high-speed all-optical switching has been demonstrated [8], and is expected to bring about a breakthrough in all-optical information processing systems [9].

In the course of recent experiments we developed all-optical logic gates, based on our fast integrated optical switching technique [10]. With the help of photopolymerization, we developed miniature, single mode Mach-Zehnder structures. The principle of logical operations is based on a reversible change of the refractive index of the bacteriorhodopsin film over either or both arms of the interferometer.

Acknowledgement
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Hofmeister Effect on the FAD Molecule Revealed by Fluorescence Lifetime Analysis in the fs-ns Range

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The ability of different salts to govern the aggregation and conformation sates of proteins and other colloids is not determined simply by the actual ionic strength. Specific ion effects have similar importance, as expressed by an empirical ordering of anions known as Hofmeister series [1]. Higher degree of aggregation is facilitated by the so called kosmotropic anions (SO_4^{2-}, F^-), while open structures are preferred in the presence of chaotropic ones (ClO_4^-, SCN^-). This phenomenon clearly has high biological importance, and indeed, it was experimentally observed mainly on macromolecules. On the other hand, the description of the Hofmeister effect in the framework of physical chemistry is still challenging. Model calculations – sometimes based on many-body quantum mechanical interactions – are obviously more feasible on simple systems, hence experimental evidences for the effect taking place on small biomolecules would be desirable. Here we report such an evidence for flavin adenine dinucleotide (FAD), a small coenzyme of crucial biological tasks.

The flavin and adenine groups of the FAD molecule could arrange in an open (planar) conformation as well as in stacked forms, characterized by long- and short-lived autofluorescence, respectively [2,3]. In this study the time-resolved fluorescence of FAD in the presence of kosmotropic and chaotropic anions was followed in the 100 fs – 10 ns region by a home-made apparatus combining the methods of fluorescence upconversion and time-correlated single photon counting. The fluorescence kinetics traces were analyzed by transforming the raw data into a population distribution on a quasi-continuous wide range of exponential time constants [3]. The transformation – applying the Basis Pursuit Denoising method – preferred sparse solutions i.e. population distributions consisting of a small number of very sharp peaks.

As we observed earlier [3] we have found that even in pure water a considerable amount of FAD molecules populated the open conformation, characterized by a peak at time constant of 2.5 ns, and the fluorescence kinetics distinguished three different stacked states, corresponding to peaks at 2 ps, 8 ps and 80 ps. The presence of the Hofmeister salts hardly changed the position of the peaks. On the other hand, the kosmotropic and chaotropic anions considerably increased the population of the stacked and open conformations, respectively, in complete accordance with the observations obtained on macromolecules.

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Functional dynamics of BLUF domain proteins revealed by ultrafast spectroscopy

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Light sensing proteins mediate the response of living systems to light. In the most widely studied examples, rhodopsins, phytochromes and photoactive yellow protein, the primary process involves an excited state isomerization reaction which converts light energy into a mechanical perturbation, leading to a structure change in the protein and ultimately to the signalling state. Relatively recently a range of blue light sensing flavoproteins have been discovered and shown to be widespread, occurring in animals, plants, fungi and bacteria [1]. Three separate classes of photoactive flavoproteins have now been identified: photolyase/cryptochromes; light-oxygen-voltage (LOV) domain proteins; blue light utilizing flavin (BLUF) domain proteins. In each case the chromophore is a flavin (isoalloxazine) ring which is planar in its oxidized form, and thus not able to exert a mechanical force on the surrounding protein.

The BLUF domain is a versatile unit involved in phototaxis in *Synechocystis* [2], biofilm formation in *Acinetobacter baumannii* [3], and gene expression in *Rhodobacter sphaeroides* [4,5], processes which are controlled by the BLUF proteins PixD (slr1694), BlsA and AppA respectively. The role for the BLUF domain in light induced regulation of gene expression means it is a candidate for exploitation in the emerging field of optogenetics.

In this work we present ultrafast transient absorption measurements in the visible and mid-infrared region as well as time resolved multiple probe spectroscopy performed on WT AppA, AppA mutant Y21W and PixD. With these methods we are able to identify the individual steps of the photocycle.

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Photosynthetic protein in biophotovoltaics - the issue of charge recombination.

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The trend in the design of biophoto-electrochemical devices is to aim for direct electron transfer between electrodes and the redox centers of the biocatalyst. The intended outcome is to achieve minimal overpotential to avoid loss of voltage in energy conversion. This strategy is ideal when direct electron transfer is fast [1,2]. However, this is not possible with all photosynthetic proteins.

Electron relays may be implemented as an efficient alternative provided their properties, and in particular their redox potentials, are tuned to enable maximal current density at low overpotential. To illustrate the desired parameters of an electron relay and of its polymeric supporting matrix, the example of bio-photoelectrochemical cells as well as a full bio-photovoltaic cell based on photosynthetic protein complexes will be given [3-5]. While the electron transfer rates between electrodes and the photosynthetic proteins have dramatically increased with recent novel immobilization strategy, the use of freely diffusing electron mediators faces the issue of charge recombination that may eventually limit their applications.

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Ultrafast light pulse sources for time resolved studies of ELI-ALPS

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The Attosecond Light Pulse Source (ALPS) facility of the pan-European Extreme Light Infrastructure (ELI) project is designed to implement a laser based research infrastructure in which light pulses of few optical cycles are generated and used for basic and applied research, especially for time-resolved studies of any kind of light-matter interactions.

The research infrastructure of ELI-ALPS is based on four main laser sources, operating with few 100W average power around 800 nm, but at different repetition rates and peak power. The variety of the primary sources is completed by a mid-infrared laser and nonlinear optical freqency conversion stages, so that the entire VIS-IR spectral range would be covered. The systems are designed for stable and reliable operation, yet to deliver pulses with unique parameters, especially of unparalleled fluxes and extreme broad bandwidths.

The high repetition rate (HR) system is designed to run with various optical parametric amplification technologies offering 1TW peak power, < 5 fs pulses at 100 kHz. The system will feed the gas high harmonic sources with CEP stabilized pulses at 1 mJ and sub 7 fs from mid 2016. The ultimate specification will be available for the users by 2018.

The backbone of the facility is the 1kHz repetition rate (SYLOS) system, which will be providing the users with pulses of 4.5 TW at sub-10 fs duration by 2016. The performance will be boosted up to 20 TW peak power at sub-5 fs, keeping the CEP stability better than 250 mrad by the end of the implementation phase, while the single cycle regime is expected to reach by the end of the decade. The SYLOS system would drive versatile secondary sources, ranging from few tens of attosecond duration, keV photon bunches generated via surface high harmonics, through high photon flux coherent soft X-ray single pulses based on gas harmonics, to low flux capillary electron sources.

The high field (HF) laser delivers 2 PW peak power optical pulses with parameters that combine ultra-high temporal contrast ($C>10^{11}$), shortest possible pulse duration (17 fs), and the highest repetition rate (10 Hz) by late 2016. The HF laser will drive novel attosecond light sources beyond keV, based on surface harmonics as well as Thomson scattering, and would also provide regional radiobiological researches with ions.

The mid-infrared laser system at 100kHz repetition rate will be operational by end 2016, providing multi-dimensional spectroscopical studies with tuneable (2.5-3.5 μm) laser pulses at ultrashor pulse duration of less than 4 optical cycles and 150 μJ energy.

The unique source parameters will enable intriguing new insight in valence and core electron science, attosecond imaging in 4D, relativistic interaction, manipulation of matter by intense THz fields, and various biological, medical, and industrial applications.

Advanced X-ray spectroscopy methods for characterization of small-molecule activating transition-metal centers in biological enzymes and catalytic materials

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Transition-metal active sites in enzymes catalyze a broad range of small molecule activation and turnover reactions at ambient conditions, which are of crucial relevance for future renewable fuel supply, industrial catalysis, and medicine. Important examples are photosynthetic water oxidation at a manganese-calcium complex, hydrogen chemistry at nickel and iron centers, and carbon-dioxide conversion at complexes containing nickel, iron, or molybdenum ions. These systems may provide blueprints also for the development of new synthetic catalysts using, e.g., biomimetic approaches.

Advanced X-ray spectroscopy methods at third or fourth generation synchrotron sources facilitate characterization of the molecular and electronic structures of complex metal centers in enzymes and synthetic materials in the course of the catalytic cycle with unprecedented site- and spin-selectivity, as well as providing high temporal and spatial resolution. The opening of new synchrotron facilities around Europe and worldwide, in particular the emerging free electron laser sources, now offer exciting perspective for novel high-resolution X-ray experiments.

Examples of our recent X-ray absorption and emission spectroscopy experiments using time-resolved approaches and site-selective methods for investigation and discrimination of metal species in sophisticated clusters in proteins will be presented. The results have provided novel insights into the restraints that govern, for example, the photosynthetic oxygen formation chemistry and hydrogen turnover in hydrogenases [1-5]. Future perspectives and applications of advanced X-ray techniques are briefly outlined.

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The structure of nature's water splitting catalyst prior to O-O bond formation: Water Binding and Water Splitting in Photosynthesis

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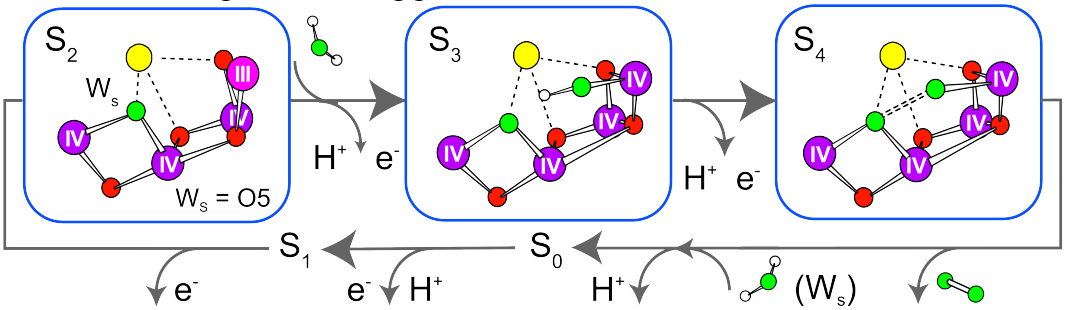
EPR spectroscopy is a versatile technique for the study of transition metal cofactors, providing chemical information on the geometric and electronic structure of the complex itself and its interaction with the substrate. Experiments performed with isotopically labelled water ($H_2{}^{17}O$/ $H_2{}^{18}O$) provide a means to identify the two substrate binding sites of nature's water splitting catalyst, a pentaoxygen tetramanganese-calcium cofactor. Earlier membrane-inlet mass spectrometry (MIMS) results, which monitor the uptake of $H_2{}^{18}O$ into the product O_2 molecule [1], have demonstrated that the complex contains two chemically different substrate sites: an early (W_s) and late (W_f) binding substrate site, both of which exchange with bulk water in all catalytic states (S-states) [1]. Owing to the relatively slow rate of exchange of W_s ($\approx 1\ s^{-1}$), and its dependence on the oxidation state of the Mn tetramer, W_s is usually considered to be an oxygen ligand of one of the manganese ions [1]. By using water labelled with the magnetic isotope (^{17}O, $I = 5/2$) the same substrate binding site (W_s) can be characterized spectroscopically, using the EPR technique, ELDOR-detected NMR (EDNMR) [2,3]. These measurements identify a unique, exchangeable μ-oxo bridge as a potential candidate for W_s. It is noted that the unusually fast rate of exchange of O5, as compared to that of μ-oxo bridges in simple synthetic model systems is likely due to the unusual flexibility of O5's metal coordination [4]. Indeed μ-oxo bridge lability appears to be a feature of new heterometallic models of the biological cofactor [5].

These results are complemented by recent multi-frequency, multi-resonance (X-, Q-, W-band) pulse EPR data obtained for the last metastable intermediate of the catalytic reaction cycle, the S_3 state [6]. It is observed that in this state all four Mn ions are structurally and electronically ***similar***: they all have the same formal oxidation state of 4+ and an octahedral local geometry ($t_g{}^3e_g{}^0$) [6]. These results are interpreted with the aid of density functional theory calculations on models developed from the recent X-ray crystal structure [6,7]. It is shown that only one structural model is consistent with all magnetic resonance data. This model requires the binding of an additional water molecule, possibly the second substrate water to the manganese cofactor during the formation of the last intermediate, and assigns its binding position.



Together these experimental results resolve the mechanism of the biological water splitting reaction, with O-O bond formation occurring between two manganese-bound oxygens in the transition state, most likely an oxo-bridge and an oxyl radical [8]. It is demonstrated that structural

flexibility is important for second substrate inclusion [4] and that oxygen-oxygen coupling is facilitated by the spin topology of the cofactor [6,8].

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Electron dynamics in molecules investigated by attosecond pulses

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The generation and characterization of single attosecond pulses have been achieved through several efforts in the field of ultrafast intense laser sources over the last 20 years [1] and through theoretical developments on the interaction of intense light pulses with atomic and molecular systems [2]. The duration of attosecond pulses is rapidly approaching the atomic unit of time [3,4] that represents, in the classical description of the atomic model, the natural time scale of the electronic motion; also in quantum mechanics the attosecond regime is the relevant time domain for electrons as the inverse of the energy spacing among electronic levels, (that determines the time constant for non-stationary states), lies typically in this range. First applications of such pulses have been mainly focused on simple atoms or molecules to validate new experimental approaches and to gain first information on electron-electron correlation.

The first experiment showing sub-cycle control of the electronic dynamics was reported in H_2 and D_2, by using the combination of a single attosecond pulse and a synchronized infrared field [5].

Theoretical models and recent experiments indicate that attosecond and few-femtosecond dynamics are relevant also for the understanding of electronic and nuclear dynamics in more complex molecules such as biomolecules. In this context, we will show first results on the coupled electronic-nuclear dynamics in caffeine molecules.

New directions for the investigation of attosecond dynamics in more complex molecular systems, and, in particular, the new scientific possibilities opened by the attosecond sources that will be available at ELI-ALPS, will be discussed.

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POSTERS

P1

Carotenoid and bacteriochlorophyll exchange approach to investigate antenna processes in bacterial LH1 complex

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The photosynthetic unit of purple bacteria is among the simplest found in nature. In *Rhodospirillum rubrum* it consists of a charge-separating reaction center surrounded by a ring-shaped LH1 antenna. LH1 is a transmembrane complex composed of 16 α–β polypeptide subunits, each hosting two bacteriochlorophyll *a* molecules and one spirilloxanthin molecule. Reaction center performs the charge separation across the photosynthetic membrane, which is a starting point of a series of redox reactions necessary for efficient conversion of light energy. In effect, the photosynthetic unit is capable of converting photons to electrochemical energy with quantum yield approaching 100%.

The modular structure of LH1 allows for its *in vitro* reconstitution from detergent dissociated subunits, and a replacement of both of its cofactors by modified bacteriochlorophylls and/or non-native carotenoids. The ground-state properties of Ni-bacteriochlorophyll *a* resemble these of the native pigment, but its excited-state lifetime is dramatically shortened. Its presence in LH1 causes an ultrafast excitation quenching and leads to fluorescence decay correlated to the extent of pigment exchange. The substitution of native spirilloxanthin with spheroidene increases overall efficiency of intracomplex singlet energy transfer, and shifts the light absorption by LH1 in the blue-green spectral region. Such modifications combined with a detailed spectroscopic analysis constitute a convenient tool for studying the mechanisms of assembly and functioning of photosynthetic antenna.

P2

Triplet state kinetics of the pigments in the light-harvesting complexes of selected algae from a SAR group

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The SAR phylogenetic supergroup is composed of Stramenopiles, Alveolates and Rhizaria. Photosynthetic members of this group acquired a plastid via a secondary endosymbiotic event between a nonphotosynthetic bicont-like protista and a red alga. Light-harvesting complexes of *Chromera velia* (chromera light-harvesting complex, CLH), belonging to Alveolata, and *Nannochloropsis oceannica* (violaxanthin-chlorophyll *a* binding protein, VCP) and *Xanthonema debile* (xanthonema light-harvesting complex, XLH), both belonging to Stramenopiles, contain only chlorophyll *a* and lack chlorophyll *c*, characteristic for other photosynthetic algae from the group. CLH and VCP-like sequences show high homology to fucoxanthin containing proteins of diatoms [1,2], while they differ in carotenoid composition. Transmission electron microscopy revealed a similarity between XLH and CHL structures [2]. In presented study triplet-triplet energy transfer in these light-harvesting complexes was investigated by combination of time-resolved fluorescence and absorption spectroscopy. The results indicate a ~100% efficiency of chlorophyll triplet quenching by carotenoids, which allows for efficient photoprotection.

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P3

Activation of Carotenoid Intramolecular Charge-Transfer State in LH1 Complexes of Various Purple Bacteria

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We have identified spectroscopic differences between LH1 and LH2 complexes of *Rhodobacter (Rba.) sphaeroides*, which bind a carbonyl carotenoid spheroidenone [1]. We conclude that these differences are caused by a specific carotenoid configuration (s-*trans*) induced by LH1 protein environment. That spheroidenone is in the s-*trans* conformation can be observed thanks to a specific band around 750 nm in the transient absorption spectra recorded after carotenoid excitation. We relate this band to the activated intramolecular charge-transfer (ICT) state. Our aim is to find structural determinants of the ICT state activation.

To this end, we have compared transient absorption spectra of LH1 complexes of *Rba. sphaeroides* with those of two other representatives of phototrophic anoxygenic bacteria containing carbonyl carotenoids in LH1, i. e. *Roseobacter* (*Rsb.*) strain COL2P (contains spheroidenone) and *Chromatium* (*C.*) *purpuratum* (contains okenone). While the 750-nm band appears in transient absorption spectra of LH1 complexes from *Rsb.* strain COL2P, no such signal has been detected in case of LH1 complexes from *C. purpuratum*. We have thus compared protein sequences of the LH1 complexes from the three species, which led to an identification of a single amino acid probably responsible for ICT state activation.

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P4

Towards a structure-based exciton Hamiltonian for the CP29 antenna of photosystem II

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The exciton Hamiltonian pertaining to the first excited states of chlorophyll (Chl) *a* and *b* pigments in the minor light-harvesting complex CP29 of plant photosystem II is determined based on the recent crystal structure [1] at 2.8Å resolution, applying a combined quantum chemical/electrostatic approach as used earlier for the major light-harvesting complex LHCII [2]. Two electrostatic methods for the calculation of the local transition energies (site energies), referred to as the Poisson–Boltzmann/quantum chemical (PBQC) [3] and charge density coupling (CDC) method [4], which differ in the way the polarizable environment of the pigments is described, are compared and found to yield comparable results when tested against fits of measured optical spectra (linear absorption, linear dichroism, circular dichroism, and fluorescence).

The lowest site energy is found to be located at Chl *a*604 close to neoxanthin. This assignment is confirmed by the simulation of wild-type-minus-mutant difference spectra of reconstituted CP29, where a tyrosine residue next to Chl *a*604 is modified in the mutant. Nonetheless, the terminal emitter domain (TED), i.e. the pigments contributing mostly to the lowest exciton state, is found at the Chl *a*611–*a*612–*a*615 trimer due to strong excitonic coupling between these pigments, with the largest contributions from Chls *a*611 and *a*612. A major difference between CP29 and LHCII is that Chl *a*610 is not the energy sink in CP29, which is presumably to a large extent due to the replacement of a lysine residue with alanine close to the TED.

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P5

Peridinin and Carbonyl Fermi Resonance, A Biophysical Study using Resonance Raman Spectroscopy

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Carotenoids represent one of the most widespread groups of naturally occurring pigments; they are largely responsible for the red, yellow, and orange color of fruits, vegetables, flowers and a wide range of animals. Characteristically, carotenoids contain alternating carbon-carbon single and double bonds whose electrons are delocalized around them. This electron cloud is responsible for their color, and also gives carotenoids their electronic properties that confer them various functions in biology, ranging from light-harvesting and energy dissipation to protection against singlet oxygen [1].

One carotenoid molecule in particular, peridinin (Per), is found in the light harvesting complexes of dinoflagellates. These water-soluble complexes, called peridinin-chlorophyll-proteins (PCPs), have received considerable attention in the last 15 years [1] given the availability of high-resolution structures from X-ray crystallography [3] and their peculiar photophysical behavior [3,4]. Furthermore, artificial PCP (with simpler structure and/or different chlorophylls) can be produced by mixing the apoprotein with exogenous pigments, making these proteins ideal systems to investigate energy transfer mechanisms.

The structure of the PCP complex consists of two symmetric domains, each with a central chlorophyll *a* (Chl-*a*), surrounded by four peridinin molecules [4]. In PCP, each Per displays different absorption properties, suggestive of different functions within the protein [3].

In this work, in order to provide a systematic approach to separately probe the peridinin molecules found in PCP, we provide an extended study of the vibrational signature of the molecule in various solvents by combining resonance Raman spectroscopy (RRS) with theoretical calculations. The presence of a Fermi resonance due to coupling between lactonic C=O stretching and the overtone of a C-H wagging mode provides a spectroscopic way of differentiation, and can be applied to other butenolides, making this work interesting for spectroscopists working with these molecules.

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P6

Functionalised hematite photoelectrode with engineered C-phycocyanin for hydrogen generation by water splitting

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Solar energy is gaining much more scientific interests recently for the increasing demand of energy. Artificial photosynthesis has been demonstrated to be a bright pathway to convert sunlight into chemical fuels. For large scale industrial application, the artificial photosynthesis devices have to be fabricated at low cost with reasonable efficiencies. The abundance and stability make Iron oxide (hematite) a promising light absorbing material for solar energy conversion [1-3]. Therefore, we will employ hematite coupling with some light harvesting proteins to enhance the efficiencies of hematite based photoelectrochemical cells to generate chemical fuels such as Hydrogen. Phycobiliproteins including C-phycocyanin play an important role in harvesting light in cyanobacteria and rhodophytes for utilization of a broad part of the solar spectrum to generate energy [4]. In our project, engineered C-phycocyanin will be produced in E. coli and immobilised on the surface of hematite deposited on a substrate at first by spin-coating as a photoelectrode. This electrode will be assessed by photoelectrochemical and x-ray/electron spectroscopy methods. As such, the working conditions of photoelectrochemical cells will also be investigated and optimized such as testing solution with different conductivity, composition and PH. The expected outcome is to obtain a hybrid photoelectrode for hydrogen generation by water splitting with acceptable performance.

Acknowledgement
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P7

Singlet Oxygen Concentration in Reaction Center/Carbon Nanotube Bio-nanocomposite

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The primary events of photosynthesis take place in the chlorophyll containing reaction center protein complex (RC), where the energy of light is converted into chemical potential. Under conditions when the RC photochemistry is oversaturated reactive oxygen species (ROS, e.g., singlet oxygen ($^1O_2$), superoxide anions ($O_2^{\bullet-}$), and hydroxyl radicals ($^{\bullet}OH$), chemicals with extremely high cyto- and potential genotoxicity) are formed with large probability [1-2]. There is a large interest to reduce the formation of the ROS components because they may reduce the efficiency of photochemical energy conversion. The aim of our work is to create a system for efficient light energy conversion (e.g. photovoltaics), integrated optoelectronic devices or biosensors (e.g. for specific detection of pesticides). We bind RC, purified from *Rhodobacter sphaeroides*, to different carrier matrices like carbon nanotubes (CNT), conductive polymers and porous silicon. One of the most determining factors for stability of the system seems to be the singlet oxygen generation accompanying the photochemistry. In our work we used 1,3-diphenylisobenzofuran (DPBF) to detect the arising singlet oxygen [3]. As our system is very complex and inhomogeneous, the singlet oxygen generation was measured under different parameters, using different binding methods, concentrations and spectral intervals [4]. The equilibrium concentration of the $^1O_2$ is a result of the rate of the forward sensitization and the backward deactivation processes. Although, CNTs are known as $^1O_2$ sensitizers, under our conditions (short time far red light illumination) it does not play much role in generation of $^1O_2$ and the main $^1O_2$ sensitizers are the carotenoid-less R-26 RCs. Carotenoids quench the triplet excited state of BChls in the RCs and/or react with the $^1O_2$ once it is formed. CNTs can be sensitized by $^1O_2$ directly, depending the band structure (conductivity, chirality, thickness, functionalization, etc.) and/or react chemically, typically through cycloaddition reactions.

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P8

Production and characterization of *Chlamydomonas mutants* for optical and electrochemical bio-sensing

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The increased concern about ecological damages and human health threats deriving by persistent water and soil contaminations boosted the emerging of the bio-sensing technologies as reliable, fast and efficient tool for large-scale and in field monitoring of various chemical species. Photosynthetic microalgae are among the most preferred microorganisms for environmental monitoring and screening of food and agricultural products for hazards compounds. The unique features and structural constituents of the photosynthetic systems make them a suitable sensing element, largely due to their ability to conduct charge separation and electron transfer sensitive to the presence of different classes of pesticides, heavy metals, some drugs and explosive compounds. However, the photosynthetic bio-recognition elements have some limitations related to inadequate stability and sensitivity, which negatively affects the biosensor performance. This work aimed at overcoming the principal bottlenecks of photosynthesis based biosensors by improving the resistance of bio-sensing element to oxidative damage and its affinity to different classes of pollutants. Novel bio-sensing elements for the detection of herbicides were generated by an *in vitro* directed evolution strategy targeted at the photosystem II D1 protein of *Chlamydomonas reinhardtii*, using exposures to radical-generating ionizing radiation as selection pressure. This approach proved to be successful in identifying D1 mutations conferring enhanced stability, tolerance to free-radicals-associated stress and competence for herbicide perception. In parallel, computational methods were exploited to identify single aminoacidic substitutions in the D1 protein conferring an increased affinity to the herbicide, atrazine, and prove of concept was achieved by studies on *de novo* generated D1-site-directed mutants. This research is focused on the various strategies for production and characterization of D1 *Chlamydomonas* mutants suitable for optical and electrochemical bio-sensing of herbicides contaminants in nanomolar concentration range.

Acknowledgement
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P9

Ultrafast Quenching of Chlorophyll Excitons in Photosystem II Antenna Coupled to Indium Tin Oxide Nanoparticles

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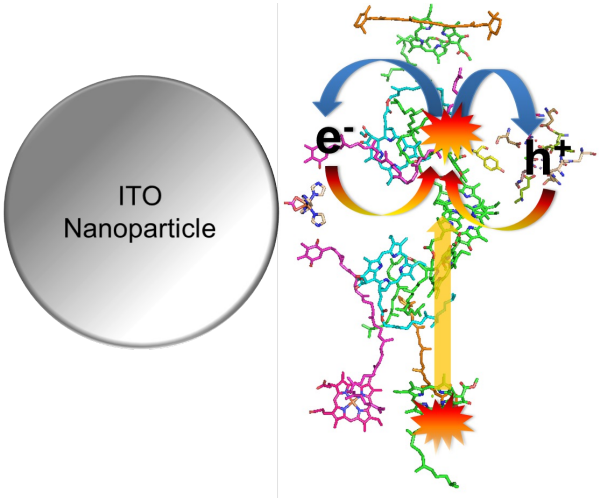
Photosynthetic proteins such as the water oxidation enzyme, photosystem II, have always been the subject of great curiosity for their subtle, yet complex exciton and charge mechanisms. Photosystem II exhibits phenomenally fast exciton transfer and charge generation/separation, whilst maintaining very low yields of recombination and decay [1-3]. As the field of hybrid photosynthetic systems, which incorporate natural enzymes for water splitting and/or fuel generation, begins to play more of a role in solar energy based research, questions have to be asked about how these natural systems really perform under such artificial conditions [4-6]. It is reasonable to hypothesize that the intrinsic photophysical processes can be perturbed due to these new environmental conditions, whether it is induced via steric and/or electronic factors. Here we present initial investigations into the exciton-charge dynamics of solution based Photosystem II coated indium tin oxide nanoparticles. This simplistic system looks to see whether the classic transient spectroscopic signatures used for kinetic studies of the natural enzyme show any evidence of perturbed intra-protein kinetics. Room temperature transient absorption and time-resolved photoluminecence spectroscopy has shown evidence for such perturbations occurring on the fs-ps time scale. Such studies could aid in elucidating morphological dependencies of inter-pigment electronic coupling, which is a known pre-requisite for high quantum efficiencies of energy and/or charge transfer.



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P10

Effects of detergents, lipids and trimer-trimer contacts on the pigment excitonic interactions in plant light-harvesting complex II

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In photosynthetic membranes fast and efficient directional excitation energy transfer occurs on account of short-range intermolecular excitonic interactions between pigments in the light-harvesting pigment-protein complexes, which give rise to characteristic circular dichroism (CD) signals. CD is highly sensitive to the excitonic interactions and to the molecular architecture of photosynthetic membranes. It is well known that detergent extraction of plant light-harvesting complex II (LHCII) from the native membrane or from aggregates brings about significant changes in the excitonic CD spectrum. To elucidate the cause of these changes, e.g. trimer-trimer contacts or detergent-induced structural perturbations, we compared LHCII aggregates, artificial LHCII-lipid membranes, LHCII trimers solubilized in different detergents, trapped in polymer gels in the absence and presence of detergents. By this means we were able to separate the spectral changes specific to protein-protein contacts (at (+)437 nm and (+)488 nm) from those due to detergent-protein interactions (e.g. at (+)446 nm and (-)495 nm). We further distinguished spectral signatures of protein-protein interactions occurring in the lipid bilayer from those in random aggregates.

The anisotropic CD (ACD) of macroscopically-aligned LHCII was employed to discriminate between excitonic transitions with different polarizations, i.e. found predominantly parallel (face-aligned) or perpendicular (edge-aligned) to the membrane plane. In line with theoretical considerations, the ACD spectra of oriented LHCII in face-aligned position exhibited only some of the bands present in the CD spectra of randomly oriented (isotropic) solution and the amplitudes of these bands were strongly amplified. On this basis, the (+)445 nm and (+)483 nm CD bands could be assigned to excitonic transitions oriented in the membrane plane and the bands at (-)437 and (-)473 nm to excitonic transitions perpendicular to the membrane plane.

Acknowledgement
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P11

Organic solar cell sensitized by photosynthetic reaction center protein

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Intensive studies have recently shown that photosynthetic proteins purified from plants (PS-I and PS-II) and from purple bacteria bind successfully to nanostructures, however their functional activity is largely retained. Current researches are focussing on finding the best bio-nanocomposite sample preparations and experimental conditions for efficient energy conversion and for the stability of the systems. In our studies reaction center proteins (RC) are purified from purple bacterium *Rhodobacter sphaeroides*. RC sensitized organic solar cell was constructed by P3HT-MWCNT-RC (P3HT: Poly(3-hexylthiophene-2,5-diyl) and MWCNT: multi-walled carbon nanotube) complex as active layer, PEDOT:PSS (PEDOT: Poly(3,4-ethylenedioxythiophene) and PSS: Polystyrene sulfonate) as anode and silver as cathode. The photochemistry and the photocurrent generation by our RC sensitized organic solar cell were measured in dry condition. Flash photolysis and current-voltage characteristics indicate that after preparing our solar cell sample the RC performs photochemical activity. Photocurrent and the photovoltage were measured even without applied potential. Moreover, the complexes have high stability and generate photocurrent in dry conditions.

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P12

Excitation dynamics in Photosystem I studied by streak-camera measurements at room temperature and at 77 K

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Photosystem I (PSI) is a large pigment-protein complex which uses the light energy to drive electron transport across the thylakoid membrane. PSI contains its own antenna system formed by ~90 chlorophyll *a* molecules bound in protein matrix. The antenna system is responsible for the light absorption and delivery of the excitation energy to the reaction center (RC), located in the central part of PSI, where the electron transport is initiated. In algae and plants, PSI is also equipped with additional light harvesting complexes (LHCI) supporting the effective light collection.

In order to investigate the excitation dynamics in PSI, a series of time-resolved fluorescence measurements were performed at room temperature (RT) and at 77 K using a streak camera setup (time resolution of ~3.5 ps). Measurements were carried out for PSI cores (i.e. PSI particles devoid of LHCI) and PSI-LHCI complexes isolated from the green alga *Chlamydomonas reinhardtii*. The detailed comparative analysis of the obtained results allowed us to determine the spectral properties of chlorophylls forming the antenna systems in PSI complexes and their impact on the observed excitation dynamics as well as to estimate the difference in the average trapping times of excitations produced in the PSI core and the LHCI antenna system.

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P13

Laser Induced Forward Transfer as an immobilization technique, for biomaterials

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This work presents the Laser Induced Forward Transfer (LIFT) us an immobilization technique, of biomaterials on sensor surfaces. The LIFT technique is well known for the printing biomaterials such us DNA [1], proteins [2] etc. and the printing mechanism relies on the irradiation of a donor substrate that caries the biomaterial in liquid form, and the generation of a jet that deposits the biomaterial on a receiver substrate. This technique has recently been used, by our group, not only for the deposition of the biomaterials on sensor surfaces, but also for the direct immobilization of biomaterials without any chemical functionalization layer [3,4].

The immobilization mechanism rely on the high travel velocity of the biomaterial from the donor substrate, to the receiver substrate, due to the laser irradiation. The range of the travel velocities that can be reached due to laser printing is between 30 to 200 m/s that lead to impact pressures from some kPa to 40 MPa. This high impact pressure of the transferred liquid on the receiver substrate, results of the physical absorption of the biomaterial on the sensor surface.

The immobilization efficiency was evaluated by fluorescent microscopy images of laser printed photosynthetic materials (thylakoid membranes). The results show that this simple, direct and chemical-linkers-free immobilization technique is valuable for several biosensors and microfluidic applications since it can be applied to a variety of substrates, leading to the selective immobilization of the biomaterials, due to the high spatial printing resolution of LIFT technique.

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P14

Monitoring the electricity production profile of Rhodobacter sphaeroides in optimized conditions

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Photosynthetic bacteria are a unique species of microorganisms that use the sun as a source of energy. The purple bacteria and green nonsulfur bacteria synthesize a nonoxygen-evolving type II photosystem. The simple non-oxygen evolving photosystem collect solar energy and convert it to chemical energy depending on photochemical reaction centers that contain chlorophylls or bacteriochlorophylls. Rhodospirillacae are purple bacteria. These bacteria can use hydrogen gas as an organic electron donor or can also use succinate or malate, depending on the availability of each compound [1].

The photosynthetic microbial fuel cell (PMFC) is a bioelectrochemical system capable of converting sunlight into electricity based on the exploitation of biocatalytic reactions within active microbial cells. In these systems, the oxidation of a carbon source occurs at the anode while the reduction of O_2 to H_2O occurs at the cathode [2].

In this study we optimized the electricity production profile of *Rhodobacter sphaeroides*, in a PMFC designed by us.

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P15

Photosynthetic reaction center structures in nanocomposite systems – what you can see with polarization spectroscopy

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Photosynthetic reaction centers (RCs) use solar light energy for the production of oxygen and organic compounds. Charge separation in the excited RC occurs with near 100% efficiency, followed by electron transport that finally stores the energy into stable chemical bonds. RCs isolated from anoxygenic photosynthetic bacteria have served as early models for studying the mechanisms of photosynthesis and are still in the focus of intensive research. Special attention has been paid recently to the potential application of protein complexes, like RCS, in hybrid systems with inorganic material as artificial solar energy convertors, biosensors, or nanoelectronic devices. Carbon nanotubes (CNTs) are under special interest of nanoparticle research. Promising new bionanocomposites could be assembled from CNTs and bacterial RCs. The composites have unique mechanical, electrical and optical properties; therefore they could be used for solving specific tasks, such as energy conversion and storage.

Biohybrid composites of single-walled CNTs with attached bacterial RCs were used as models to study the interaction between their components, since the structure and function of the biological material might be altered after attachment to the CNT. The spectroscopic characteristics of the attached RCs were probed by circular and linear dichroism spectroscopy (CD and LD). LD was used as to determine the orientation of the RCs with respect to the CNT geometry and CD was used to characterize the intactness of the RCs and their capability for photoinduced charge separation.

PRESENTATIONS OF TRAINEES

Study of excited-state kinetics of photosystem I and II in intact spinach leaves by picosecond fluorescence lifetime measurements

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When plants are exposed to excess light, more excitations are created in the pigments than the reaction centres of the plant cell can handle. Because this can be harmful to the cell, it has to get rid of this excess energy. One of the easiest ways to dissipate energy is by heat through non-photochemical energy (NPQ) [1]. Heat dissipation in plants cannot be measured directly, but can be studied by following the decrease of chlorophyll fluorescence (NPQ).

We report on the comparison between the excited-state kinetics of photosystem I (PSI) and photosystem II (PSII) in spinach leaves *in vivo* at different actinic light intensities. To understand the kinetics of the early steps in photosynthesis and its photo-protective mechanism, we use non-invasive picoseconds fluorescence measurements on intact spinach leaves.

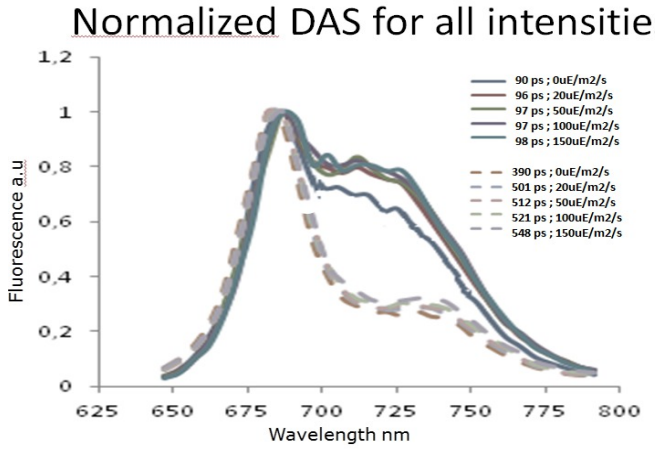

Fig 1. Normalized decay associated spectra (DAS) for different actinic light intensities.

Picosecond fluorescence measurements have been performed with a streak-camera setup and the data have been analyzed by global analysis [2]. Intact spinach leaves were placed between two glass plates in a rotating cuvette which also moves horizontally. Fluorescence kinetics has been measured for the upper surface of the leave at different actinic light intensities, ranging from 0 μE/m^2/sec up to 150 μE/m^2/sec. Figure 1 shows the fluorescence decay associate spectra (DAS) of intact spinach leaves under different actinic light conditions (2 components were sufficient to describe the fluorescence at all wavelengths). The 90 ps DAS corresponds largely to PSI and partly to PSII, whereas the 2nd DAS component ranging from 390ps (0 μE/m^2/sec) to 548ps (150 μE/m^2/sec) corresponds to PSII. These results will be discussed on the poster.

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Design principles and efficiency of light harvesting complexes in green bacteria. A picosecond fluorescence study.

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Energy transfer is the initial step in photosynthesis which drives other processes that eventually transform the light energy into chemical energy. Understanding energy transfer is important for the production of biofuels and to improve the efficiency of solar cells. The aims of the present study are to characterize light harvesting in Chlorobaculum tepidum, to obtain an understanding of how energy is transferred, and to elicit general features which are possibly applicable to other systems.

C. tepidum uses chlorosomes as its antenna complexes. Chlorosomes are made up of hundreds of thousands of bacteriochlorophylls (BChls), BChl c in the case of C. tepidum. These bacteriochlorophylls self-assemble into large layered cylindrical aggregates for which no protein scaffold is needed. The excitations are transported via a pool of lower-energy BChl a pigments in the chlorosome base plate and in Fenna-Matthews-Olson antenna complexes to the reaction centers.

We have used picosecond fluorescence measurements to study the excitation energy transfer (EET) and the trapping in intact Chlorobaculum tepidum cells. EET from BChl c to BChl a occurs on a time scale of 50-60 picoseconds whereas the overall average trapping time (charge separation in the reaction centers) is around 300 ps, corresponding to a quantum efficiency of 90-95%. It is argued that in the absence of the intermediate BChl a pigments, the chlorosomes would become hopelessly inefficient and the light-harvesting design principle of these bacteria is directly applicable to artificial photosynthesis systems and photovoltaic cells.

Interaction between *Rhodobacter sphaeroides* reaction centers and TiO_2

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Nowadays one can observe intensified development in field of alternative sources of energy, which is caused by the depletion of fossil fuels. One of the most promising is the solar energy. The invention of Dye Sensitized Solar Cells (DSSCs) by Michel Graetzel was a breakthrough in photovoltaic cells technology. One of the proposed modifications of DSSCs is replacing dyes with photosynthetic reaction centres (RCs) from different organisms, e.g. purple bacterium *Rhodobacter sphaeroides* [1]. RCs may be treated as solar cells in nanoscale. First steps of photoreaction in photosynthetic reaction centres are absorption of photon and charge separation between chromophores [2]. Process of producing described construction consists of positioning protein on the titanium dioxide porous layer. This binding is mainly based on electrostatic interactions, which means that ingredients of protein solution may have great impact on it [3].

The aim of the research was to optimize conditions of attaching proteins to the TiO_2. Additional objective was to determine the possibility of obtaining a stabilized TiO_2 solution, native to a reaction centre. For this purpose Fluorescence Correlation Spectroscopy (FCS) of RCs with TiO_2 nanoparticles in solution was performed. By using this method one can obtain information about the size of the measured complex of RCs and titanium dioxide.

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Investigation of plasmonic nanostructures for multiphoton spectroscopic applications based on pH-sensing

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Modern laser spectroscopic methods in combination with microscopy provide a promising and exciting possibility to explore complex structures and systems in the environmental and life sciences. In recent methodological developments, multi-photon and nonlinear spectroscopies have been given high priorities due to several advantages. First, nonlinear light−matter interaction in multi-photon processes follow different selection rules than the corresponding one-photon processes, therefore, they can deliver complementary spectroscopic and structural information. Second, for biological objects, nonlinear spectroscopy offers advantages mainly due to excitation with lower photon energy and reduced excitation volumes (in the low fL range) compared to linear techniques.

Multiphoton vibrational spectroscopy, such as techniques based on hyper Raman scattering, (HRS) can reveal local molecular structural information about complex biosystems. In microscopies, local fields obtained by localized surface plasmons play an increasingly important role and have pushed the research field of plasmonics. Spectroscopic and non-linear optical responses can be strongly affected by the local near-field of highly versatile metallic nanoparticles (NPs). For example, in surface enhanced HRS, resonances between surface plasmons and optical fields lead to strongly enhanced signals because of the nonlinear dependence of the scattering intensity on the excitation field [1]. Due to the expected huge potential of plasmonic NPs in bio-applications, the investigation of their nano-bio-interactions and applicability to nonlinear spectroscopy is very important for fields ranging from imaging and medical diagnostics to bio-sensing or light energy conversion.

In this contribution, we compare one- and two-photon excited non-resonant Raman spectra of *para*-mercaptobenzoic acid (*p*MBA) in the local fields of various silver nanostructures in the pH range of 2-12. The vibrational modes of *p*MBA change upon protonation and deprotonation which makes *p*MBA a useful pH-nanosensor [2]. We contrast the one-photon excited spectra at 532 and 1064 nm with the corresponding two-photon excited spectra at 1064 nm regarding the possibility to distinguish different local pH-values on the basis of changes in the relative signal intensities. Multiphoton pH sensors based on *p*MBA on Ag NP can be used with one- and two-photon excitation in the NIR wavelength range. Nonlinear excitation together with the tunable optical properties of plasmonic NPs opens up new possibilities for microscopic bio-sensing and bio-photonics.

Acknowledgement
We thank Dr. Virginia Merk and Sebastian Fredrich for providing Ag ($NaBH_4$ / citrate) nanoparticles and Sören Selve for help with TEM. We also thank Dr. Harald Kneipp for discussion. Funding by ERC Starting Grant no. 259432 (MULTIBIOPHOT) is acknowledged.

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Photosystem II Thermoluminescence and Oxygen Evolving Activity in Isoprene-Emitting and Non-Emitting Tobacco Leaves and Isolated Thylakoids Before and After UV-B Irradiation

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Thermoluminescence emission from transgenic tobacco (*N. tabacum* L.) model plants including azygous isoprene non-emitting (INE) tobacco lines and homozygous isoprene-emitting (IE) lines was registered before and after short-term UV-B (10 Wm^{-2}) and after 24 and 48 hours recovery periods after the irradiation. In IE unstressed leaves the thermoluminescence B band temperature peak position was always upshifted compared to INE, indicating the more stable stored charge pairs. Isoprene was found to act with a protective role in various environmental stress conditions by strengthening the cellular membranes, thus maintaining the thylakoid-embedded photosynthetic apparatus, and/or by deactivating reactive oxygen species, thus reducing oxidation of the membrane structures [1]. The UV-B part in the solar spectrum is characterized with high potential to damage photosynthetic function. We ascertained an increased resistance of leaf photochemistry in IE plants to UV-B. If the observed protective effect *in vivo* is preserved in isolated thylakoids, was examined by comparing the oxygen evolution activity and thermoluminescence characteristics between IE and INE samples.

Acknowledgement
The authors thank Dr. Claudia Vickers for the providing with tobacco lines seeds. V.N.P. was supported by COST TD1102 action travel grant.

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In vitro analysis of the plastid terminal oxidase in photosynthetic electron transport

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The plastid terminal oxidase (PTOX) is a plastoquinol:oxygen oxidoreductase and shares structural similarities with alternative oxidases. In plants PTOX is attached to the thylakoid membrane and it is important for plastid development and carotenoid biosynthesis. Its role in photosynthesis is controversially discussed. Here a homogenously pure MBP fusion of PTOX was investigated. The protein forms a homo-tetrameric complex containing 2 Fe per monomer and catalyzes the reduction of oxygen to water as main reaction, but side reactions leading to reactive oxygen species (ROS) formation may occur. Recombinant PTOX was reconstituted with PSII enriched membrane fragments (BBYs). Addition of PTOX to a BBY sample lead to a deceleration of fluorescence induction and lowered the fluorescence maximum. By washing experiments the attachment of PTOX to the membrane was demonstrated. Investigation of ROS formation by PTOX in the reconstituted system with BBYs showed that PTOX produces superoxide anion radicals when the substrate concentration was limiting. Secondly, ROS production was investigated in the absence of PSII in a biphasic (liposomal) system in which decyl-plastoquinone (DPQ) was reduced with DT-diaphorase. PTOX did not produce ROS under optimal substrate concentrations, but at pH 6 ROS generation was observed under limiting substrate concentrations and at pH 8 when substrate was given in access. The physiological relevance of these observations will be discussed.

Removal Of Ni(II) From Aqueous Solutions By Strong Cation Exchange Resin, Amberlyst-15 (H^+)

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Removal of heavy metals from water is obligatory in order to avoid water pollution. In the present study, performance of macroporous strong cation exchange resin Amberlyst-15(H^+) was evaluated for the removal of nickel (Ni) from aqueous solutions. The adsorbent was characterized by XRD, SEM/EDX, FT-IR, TGA and surface area analyzer. The point of zero charge was in the range of 2. Batch shaking adsorption experiments were performed in order to examine the effects of pH, temperature, dosage of the resin and contact time on removal process. Pseudo–first and pseudo–second order models were used to explain the kinetic data. Equilibrium data was explained with the help of Langmuir and D-R models. Various thermodynamic parameters (ΔG, ΔS and ΔH) from nickel exchange on the resin were calculated. The negative values of Gibbs free energy suggesting the adsorption of Ni(II) onto Amberlyst-15 is thermodynamically feasible and spontaneous in nature. The positive value of ΔH implies that the adsorption of Ni(II) onto Amberlyst-15 consists of two process (a) the detachment of H^+ from the adsorbent surface and (b) the attachment of Ni(II) ions to the surface active sites of the adsorbent which points toward the endothermic nature of the adsorption process. Furthermore, the positive value of ΔS indicates that the randomness at the adsorbent surface after adsorption is increased which suggests that Amberlyst-15 has a strong affinity for Ni(II) during the adsorption process.

Microfluidic gradient generators for measuring bacterial chemotactic response

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Microfluidics is a great tool to create well-defined engineered heterogeneous environments for cellular studies. The technology is suitable for precise manipulation of liquids in microscopic dimensions and it offers possibility to detect responses of single cells or populations. Among the microfluidics-based biosensing systems, cell-based systems are probably the most recognized and often applied ones due to the potential applications in medical diagnostic, food safety or environmental monitoring. Cell-based biosensors with intact living cells as the sensor may exploit the tactic behaviour of cells. Bacteria are good candidates in such a system owing to their quick response to various external stimuli: changes in the light intensity, electric field, temperature, pressure, concentration of oxygen and other chemicals.

By chemotaxis bacteria constantly detect the concentration gradient of chemoeffectors and make „decisions" on the net direction of movement: e.g. in the presence of a gradient of attractant, bacteria bias their swimming direction towards the highest concentration of attractant. In our laboratory we have fabricated and experimentally characterized a microfluidic system that creates temporally stable chemical concentration gradient in a flow-free environment, and within the device we are able to rapidly measure the chemotactic response of bacteria.

The device is fabricated of poly(dimethylsiloxane) using photolithography and soft lithography. It consists of two large reservoirs and a narrow observation channel, separated by a porous membrane. Diffusion of molecules from the reservoirs to the central channel (where bacteria swim) creates the gradient across the channel. Although the gradients established in this case are less steep than in the popular flow based devices, they are more than enough to observe bacterial chemotaxis.

We have characterized the chemical gradients established in the device using the fluorescent dye pyranine, and fluorescence microscopy. We have studied the chemotactic response of *E. coli* to several substances. We tested some well-known attractants and repellents, such as L-aspartate and $NiSO_4$. We also measured the effect of „conditioned" media, cell-cell signaling molecules and even some antibiotics. Furthermore, by changing the buffers in the reservoirs the response of cells to altered conditions may be investigated, as well.

One of the main advantages of our device is that cells may be exposed to the gradient for extended period of time, so the behaviour of the same population can be observed for a long time (1-2 days). We are able to detect how cells change the chemical composition of their environment (consuming nutrients and releasing metabolites) and how they react to these changes on such long timescales. We were able to observe attractive and repulsive interactions between bacterial populations (*E. coli* and *P. aeruginosa*) and showed that chemotaxis and likely intercellular signalling play a fundamental role in these phenomena [1].

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Fluorescence based methods for selection of highly efficient photosynthetic apparatus for bio-sensors/biochips application

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Fluorescence emissions of chlorophyll as well as of epidermal constituents are well established reporter signals of plant stress [1]. Some of the plant stress symptoms can also be found in spectrally-resolved reflectance signals. A new combinatorial technique based on time-resolved chlorophyll fluorescence imaging was developed that can discriminate the species of the same plant family *Lamiaceae* [2], measure cold tolerance of natural accessions of *A. thaliana* [3]. We propose to use the newly developed technique to screen tolerance of "photoinhibition" [4] in differential photosynthetic apparatus of higher plants. An overview of this method and its potential application for selection of highly efficient photosynthetic apparatus in perspective of bio-sensing research will be presented. A snap of very recent results where we observed differential response of photoinhibition in the green algae having differential growth rate will be presented.

Acknowledgement
KBM thanks for the support from COST project TD1102 through STSM project to work in ESA lab in Turku.

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Construction of Solar Cells Based on *Rhodobacter sphaeroides* Reaction Centers and TiO_2

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One of the biggest problems of the contemporary world is the depletion of fossil fuels. Among the possible solutions for it, solar cells based on photosynthetic reaction centers (RCs) of purple bacterium Rhodobacter sphaeroides are considered. They have been widely used to study energy and electron transfer in RCs [1]. First steps of photoreaction in photosynthetic reaction centers are absorption of photon and charge separation between chromophores. There are some results suggesting that this process can be used to produce electricity from solar light by binding reaction centers to TiO_2 porous layer [2,3]. This construction is similar to Dye Sensitized Solar Cells (DSSC) invented by Michael Graetzel, but there proteins are used instead of dyes. Process of attaching proteins to the metal oxide semiconductor layer is based mainly on electrostatic interactions [4], so it is important to optimize conditions which influence surface charge of either protein or TiO_2.

During the presentation, results of the research on optimization of binding process and preliminary be discussed. Some preliminary results of photocurrent measurements will be also presented. Studies were conducted on wild type reaction centers and various mutants including those containing special TiO_2-binding amino acids sequence. Some stationary absorption spectra of RCs on TiO_2 and electron micrographs of the surface will be shown.

Acknowledgement
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A new triad donor-acceptor compound: synthesis and photophysical behaviour

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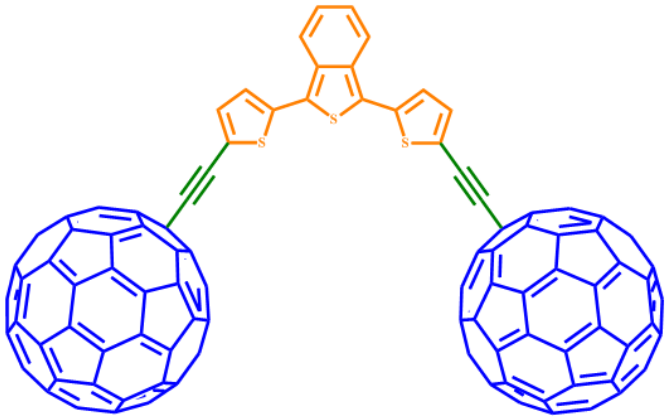
A new synthetic pathway has been recently developed for getting a donor-acceptor π-conjugated oligothiophene-fullerene C_{60} triad compound in which the three constituents are linked covalently by two ethynyl bridges (see fig) [1].



The preliminary photophysical steady-state (absorption and emission) and pump-probe techniques in solution allow us to define an electron transfer process from the electron donor DTBT moiety to the electron acceptor C_{60} counterparts. This assumption is supported by our preliminary theoretical calculations [1]. Further work is still in progress on TD-DFT theoretical calculations both on the donor-acceptor molecule and on its oligothienyl donor precursor. We aim to achieve, in such a manner, a deeper understanding on the electronic structure of the triad system as well as the mechanisms of the hypothesized electron transfer process.

Following our preliminary experimental results, further experimental evaluations (fluorescence lifetime spectroscopy) are going to be performed in order to evaluate the parameters affecting the occurrence of such electron transfer phenomena.

Based on the results obtained so far we propose that the donor-acceptor molecule designed and synthesized could be a good candidate for organic solar cells devices.

Acknowledgement
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Transport Properties Of Self-Assembled Porrphyrins & C60 Nanorods

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Self-assembly phenomenon is a pillar characteristic of various natural systems which is currently becoming a trend within the biomimicry and nanosciences fields. The approach has been explored to construct systems that mimic photosynthesis as well [1]. Artificial porphyrins and fullerene (C60) based systems are typical building blocks favorable to self-assembly processes. Coupled to such 1-D architecture, the optical and transport properties of the self-assembled porphyrins and C_{60} nano-rods/nanotubes can be ideal light harvesting 1-D type antennas. This contribution reports on the formation as well as the optical properties within the solar spectrum of $[H_4TPPS_4]^{2-}$ and $[SnTPyP]^{2+}$ based 1-D aggregates as well as C60 nanorods by the so called Miyazawa laser liquid-liquid interfacial precipitation [2].

Acknowledgement
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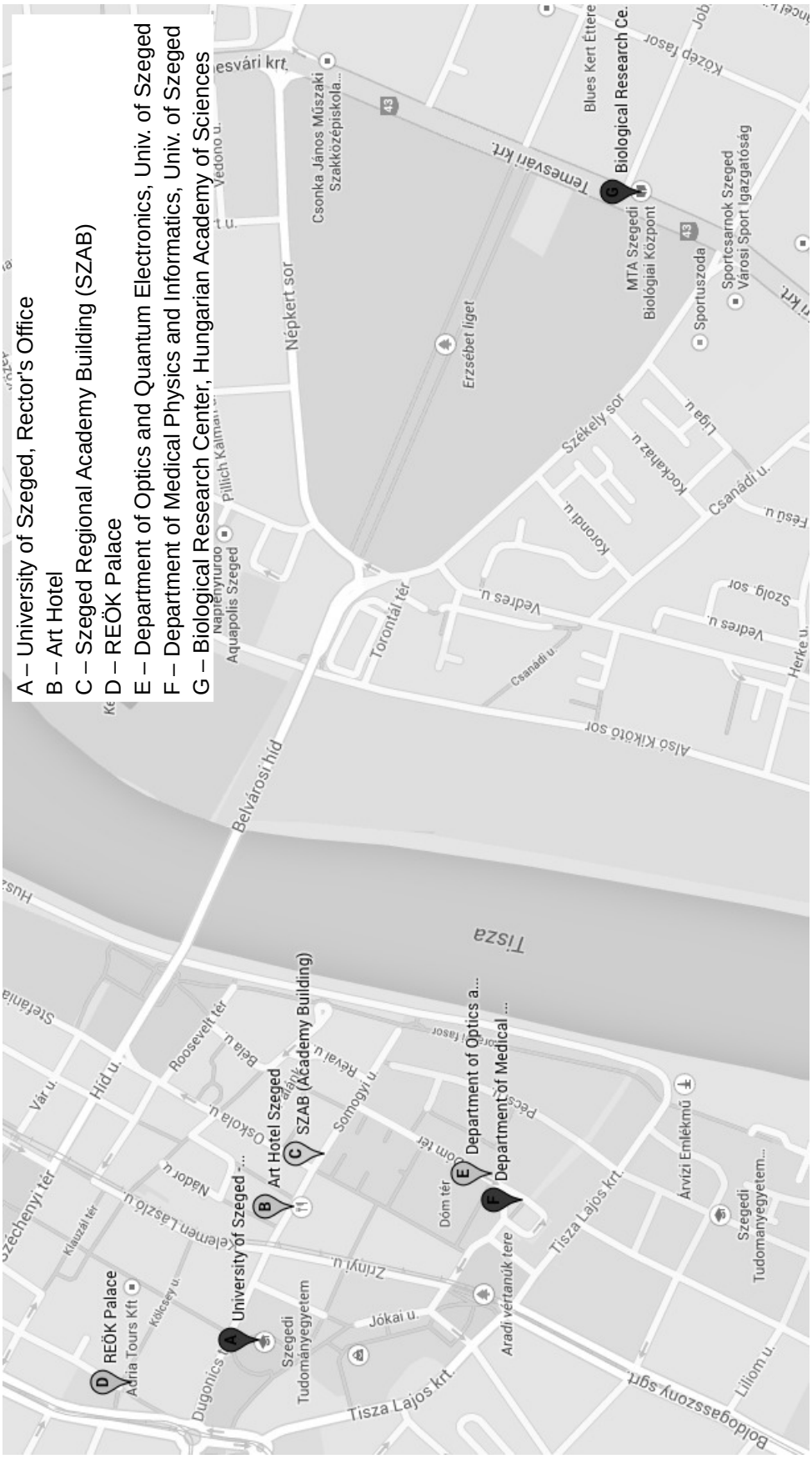
NOTES

Map – Sites of the Training School



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Time	Oct 18 (Sat)	Oct 19 (Sun)	Oct 20 (Mon)	Oct 21 (Tue)	Oct 22 (Wed)	Oct 23 (Thu)	Oct 24 (Fri)
7		Breakfast	Breakfast	Breakfast	Breakfast	Breakfast	Breakfast
8							
9		**Primary Processes of Photosynthesis** Rienk van Grondelle Richard Cogdell Roberta Croce	**Light harvesting complexes** Alfred Holzwarth Herbert van Amerongen Arvi Freiberg	**Ultrafast Photobiological Processes** András Dér Géza Groma András Lukács Nicolas Plumeré Károly Osvay	**Laboratory Practicals**	**Trainees' presentations and discussions** Shazia Farooq Parveen Akhtar Dominik Lindorfer Reza Ranjbar Choubeh Wojciech Giera Elizabeth Kish	**DEPARTURES**
9.30							
10							
10.30		**COFFEE BREAK**	**COFFEE BREAK**			**COFFEE BREAK**	
11		**XFEL. Photosynthetic Protein Complexes** Gyula Faigel János Hajdú Jan Kern	Tomas Polivka Leonas Valkunas Howe-Siang Tan Andrius Gelžinis	**COFFEE BREAK**		Marianneza Chatzipetrou Melania Kujawa Nicholas Paul Zsuzsanna Heiner	
11.30				**Monitoring Charge Movements** Michael Haumann Nicholas Cox Giuseppe Sansone			
12							
12.30							
12.45		**LUNCH**	**LUNCH**	**CLOSING REMARKS**	**LUNCH**	**LUNCH**	
13							
13.30				**LUNCH**			
14	**REGISTRATION**	Mike Jones Huub de Groot Thomas Renger Fabio Polticelli	Leszek Fiedor Krzysztof Gibasiewicz Raoul Frese Petar H. Lambrev Franz-Josef Schmitt Jevgenij Chmeliov	**DEPARTURES (Workshop participants)** **Laboratory Practicals (COST Training School)** **MANAGEMENT COMMITTEE MEETING (COST PHOTOTECH)**	**Laboratory Practicals**	Violeta Peeva Kathleen Feilke Rabail Razzaq Krisztina Nagy	
14.30							
15							
15.30						**COFFEE BREAK**	
16						Kumud B. Mishra Rafał Białek Ambra Guarnaccio Ntevhe Thovhogi Jianjun Wang	
16.30		**COFFEE BREAK**	**POSTER SESSION AND COFFEE BREAK**				
17		**Vibrational Dynamics and Raman Spectroscopy** Bruno Robert Miroslav Kloz Yang Zhao					
17.30	**OPENING ADDRESSES**						
17.45							
18	**COST PHOTOTECH ELI-ALPS** Giuseppina Rea Lóránt Lehrner Dimitris Charalambidis						
18.15			**DINNER**				
18.30		**DINNER**					
19				**Free Evening**	**Free Evening**	**CLOSING CEREMONY/DINNER**	
19.30	**WELCOME RECEPTION (buffet dinner)**						
20							
20.30		**Terahertz Spectroscopy** János Hebling József Orbán	**CULTURAL PROGRAM**				
21							
21.30							

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Szeged Regional Academy Building (SZAB)

REÖK Palace

Laboratory Practicals:

Department of Optics and Quantum Electronics, University of Szeged (1)
Department of Medical Physics and Informatics, University of Szeged (2)
Biological Research Center, Hungarian Academy of Sciences (3, 4, 5)