INTERNATIONAL CONFERENCE

TOWARDS A PHOTOSYNTHESIS-BIOBASED ECONOMY

ROME
7-9 OCTOBER 2015

Final Conference of the COST Action TD1102
Photosynthetic proteins for biotechnological applications: biosensors and biochips
ORGANIZERS
National Research Council, Institute of Crystallography, CNR-IC
Department of Bio-Agrofood Sciences (DiSBA)

CONFERENCE CHAIRMAN
Giuseppina Rea, CNR-IC

SCIENTIFIC COMMITTEE
Simona Carmen Litescu - ROMANIA
Fabio Polticelli - ITALY
Nicolas Plumeré - GERMANY
László Nagy - HUNGARY
Esa Tyystjärvi - FINLAND
Maya D Lambreva - ITALY
Giuseppina Rea - ITALY

LOCAL ORGANISERS
Amina Antonacci - CNR-IC
Viviana Scognamiglio - CNR-IC
Francesca Vergari - CNR-IC
Federica Tenaglia - DiSBA
Licia Patitucci - DiSBA

GRANT HOLDER
Institute of Crystallography, CNR, Italy
Department of Chemical Sciences and Material Technologies (DSCTM)
Legal Representative, Michele Saviano
Grant Manager, Maya D Lambreva
Financial Officer, Francesca Vergari

WEB SITE
http://www.phototech.eu
Dear participants,

It's my pleasure to welcome you all to the final conference of the COST ACTION TD1102 Photosynthetic Proteins for Technological Application: Biosensors and Biochips that is held at the CNR Headquarter from 7 to 9 October, 2015 in Rome.

The COST ACTION TD1102 aimed to explore, interface and merge the various aspects of the development of photosynthetic protein-based bio-devices with a focus on biosensors, that provide a complementary innovative tool with respect to traditional analytical methods. The main outcome was the development of a fully characterized class of bio-organic-inorganic hybrid biosensors to be first applied in environmental monitoring and agri-food quality analysis. Besides, the scientific focus of the Action TD1102 throughout the years has expanded from the development of photosynthesis-based biosensors to include research on new reliable and renewable sources for light harvesting to be utilized to meet forthcoming energy demand.

The theme of this final COST conference is hence Towards a photosynthesis bio-based economy and aims to showcase achievements of our Action and explore future opportunities in bio-energy and biofuel production. The Conference will cover basic and applied aspects of exploiting photosynthetic microorganisms or their photosynthetically active pigment-protein assemblies for the production of bio-opto-electronic devices, a new scientific and technological field with significant industrial and social impact in support of a bio-based economy. Scientific sessions will address, but will not be limited to, fundamental knowledge on photosynthesis, biosensors, biofuels, bio-photovoltaics, bio-electrochemistry, bio-hybrid solar cells.

The highly multidisciplinary Agenda of the meeting includes 4 plenary lectures by outstanding keynote speakers, 8 invited talks by leading international experts, and 18 speeches by delegates of COST ACTION TD1102, selected from submitted abstracts. The program will include also an Early Stage Researcher session with 12 oral presentations and a poster session. The Management Committee and Working Group meetings will close the event.

I wish to thank all the people of my research team and the key ladies of the department of Bio-Agrofood sciences that with their extraordinary organizational capacity, patience and effort have made this conference possible.

Again, welcome and experience a fruitful exchange of knowledge!

Giuseppina Rea

Chairman of the Conference
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Information</td>
<td>2</td>
</tr>
<tr>
<td>Conference agenda</td>
<td>3</td>
</tr>
<tr>
<td>Invited Lectures</td>
<td>9</td>
</tr>
<tr>
<td>Oral presentations</td>
<td>15</td>
</tr>
<tr>
<td>Early Stage Research Oral presentations</td>
<td>43</td>
</tr>
<tr>
<td>Posters</td>
<td>57</td>
</tr>
<tr>
<td>Authors index</td>
<td>73</td>
</tr>
</tbody>
</table>
GENERAL INFORMATION

DINING All meals will be served in Nuova Sala Catering Room. Meals will be served on the following schedule: Coffee break: 11:00 – 11:30, Light lunch: 13:00 – 14:30, Coffee break: 16:30 – 17:00. On Friday the lunch will be free.

TALKS All oral presentations will be held in Marconi Room. Please bring your presentation to the location to be uploaded onto a computer during the break before your session starts.

POSTERS Posters session will be held in Nuova Sala Catering Room on Friday afternoon. Poster board display areas are 70x100 cm and will be numbered. Please bring your poster to the location during the scheduled breaks in the morning.

MC MEETING On Friday afternoon a parallel session will be held in Giacomello Room for Management Committee and Working Group meetings.

WiFi Computer connection to the CNR WiFi network is available using the login procedures provided by local organizers.
CONFERENCE AGENDA
CONFERENCE AGENDA
PHOTOTECH 2015: Towards a photosynthesis-biobased economy
Rome, 7-9 October 2015

7 October 2015

8:00  Registration
9:30  Openings  G. Rea, Chair of the Cost Action PHOTOTECH
         M. Saviano, Director of the Institute of Crystallography, CNR
         F. Loreto, Director of Department of Bio-Agrofood, CNR

MORNING SESSION – Room *Marconi*

**Chairman: Giuseppina Rea; Gyozo Garab**

9:40  *Invited lecture: Mattoo AK.* The enigmatic D1 photosystem-II reaction center protein stands tall: Central to programming plant response to environment.


10:50  Croce R. Molecular switches in the thylakoid membrane.

11:15  **Coffee break**

**Chairman: Anja Krieger-Liszkay; Stefano Caffarri**

11:45  Tyystjärvi E. Temperature dependence of photoinhibition and singlet oxygen production.

12:10  Krieger-Liszkay A. The plastid terminal oxidase PTOX as a safety valve under excess light.

12:35  Allahverdiyeva Y. Characterizing terminal oxidase and flavodiiron electron sink function in the cyanobacterium *Synechocystis* sp. PCC 6803.

13:00  **Light lunch**

AFTERNOON SESSION – Room *Marconi*

**Chairman: Roberta Croce; Michael R. Jones**


15:40  Polticelli F. Bioinformatics approaches to Photosystem II structure, function and engineering.

16:05  Mezzetti A. Static and time-resolved infrared difference spectroscopy:
from the atomic level study of photo-induced electron transfer in isolated reaction centers to real-time monitoring of photosynthetic reactions in vivo.

16:30  

Coffee break

Chairman: Alberto Mezzetti; Esa Tyystjärvi

17:00  

Gibasiewicz K. Modeling of P^+H_2^− charge recombination in Rhodobacter sphaeroides reaction centers on the basis of both absorption and fluorescence time-resolved studies.

17:25  

Boyaci IH. Detection of herbicide using chloroplast-herbicide interaction by Raman spectroscopy.

17:50  

Klem K. Detection of carotenoids biosynthesis, PPO and ALS inhibiting herbicides using Raman spectroscopy and chlorophyll fluorescence.

18:15  

Masojídek J. Photosynthesis monitoring to estimate growth in microalgae cultures: chlorophyll fluorescence techniques.

18:40  

Grand Holder’s Document’s collection

19:30  

Conference Dinner

8 October 2015

MORNING SESSION – Room Marconi

Chairman: Raoul N. Frese; Artur Braun*

9:00  

Invited lecture: Artero V. Photocatalytic H2 evolution: from homogeneous systems to photoelectrode materials.

9:45  

Plumeré N. Photosynthetic proteins for light energy conversion into electricity and solar fuels.

10:10  

Friebe VM. Plasmonic enhancement of photocurrent generation and stability of bio-hybrid nanostructured pigment-protein silver electrodes.

10:35  

Braun A. The bio-electrochemical interface of semiconductor photoelectrodes functionalized with light harvesting proteins and cyanobacteria.

11:00  

Coffee break

Chairman: Nicolas Plumeré; Chanoch Carmeli

11:30  

Carmeli C. A biological source for efficient spin-polarized electrons- the Photosystem I.

11:55  

Grabchev I. Synthesis of a new 1,8-naphthalimide based PAMAM-type Dendron and investigating its potentiality for artificial light-harvesting.

12:20  

Milano F. Covalent attachment of photosynthetic reaction centers to hydrogen-bonded organic semiconductors for bio-optoelectronic
applications.

13:00 **Light lunch**

### AFTERNOON SESSION – **Room Marconi**

**Chairman: Daniela Russo; Fabio Polticelli**

14:30 *Invited lecture:* Pieper J. Structure and dynamics of photosystems investigated by neutron scattering techniques.

15:15 **Russo D.** From whole cells towards photosynthetic reaction centres: “functional” and intrinsic dynamic properties.

15:40 **Giardi MT.** Photosynthetic based-biosensor and applications of new strategies from basic research to market.

16:05 **Jones MR.** Engineering of the *Rhodobacter sphaeroides* reaction center for self-directed protein-electrode and protein-protein interactions in biohybrid devices.

16:30 **Coffee break**

**Chairman: Simona C. Litescu; Francesco Milano**

17:00 **Hajdu K.** Redox interaction in RC/PSiMc bio-nanocomposite.

17:25 **Giotta L.** Laser induced forward transfer (LIFT) functionalization of screen-printed electrodes with bacterial reaction centres for mediatorless herbicide biosensing.

17:50 **Trotta M.** Artificial photoconverters using genuine natural components.

18:15 **Litescu SC.** The use of electrochemical transduction in developing PSII based biosensors-pro’s and con’s.

18:40 **Janssen PJD.** Green organisms to the rescue: the potential of cyanobacteria, algae, and higher plants for the remediation of radioactively contaminated waters.

### 9 October 2015

**MORNING SESSION – **Room Marconi**

**Chairman: Ioanna Zergioti; Paul J.D. Janssen**

9:00 **Cadirci BH.** *Rhodobacter sphaeroides* converts light into electricity in a new designed microbial fuel cell by acetate photo-fermentation.

9:20 **Delgado D.** Comparing photocurrents from spinach and cyanobacteria on a conductive surface.

9:40 **Stieger KR.** Different approaches for contacting photosystem I with electrodes for efficient photocurrent generation.

10:00 **Zhang JZ.** Wiring Photosystem II to hydrogenase for photoelectrochemical water splitting.
10:20 **Kujawa M.** Binding of Rhodobacter sphaeroides reaction centres to TiO$_2$ nanoparticles.

10:40 **Magyar M.** Polarized light spectroscopy of reaction centers from *Rhodobacter sphaeroides*.

11:00 *Coffee break*

**Chairman: László Nagy; Krzysztof Gibasiewicz**

11:30 **Antal TK.** Prolonged hydrogen photoproduction by immobilized S-deprived *Chlamydomonas reinhardtii* cells: effects of light intensity, spectra, and initial medium pH.

11:50 **Stapf S.** Redox hydrogel design for (Photo)bioelectrocatalytic hydrogen evolution.

12:10 **Touloupakis E.** Scale-up of cyanobacterial cultures for photobiological hydrogen production.

12:30 **Akhtar P.** Excitation energy transfer in model photosynthetic membranes.

12:50 **Mishra KB.** Photoinhibition experiments in natural accessions of *Arabidopsis thaliana* suggest potential for improvement in the life time of PSII based bio-devices.

13:10 **Massaouti M.** Laser induced forward transfer: a novel technique for printing biomaterials.

13:30 *Closing remarks*

14:00 *Free Lunch*

**AFTERNOON PARALLEL SESSIONS**

**POSTER EXHIBITION – Room Nuova Sala Cathering**

15:00 *Fixing of posters*

15:30 *Poster Presentations*

18:30 *Closing of the Session*

**MC/WORKING GROUP MEETINGS – Room Giacomello**

15:00 *MC Meeting*

16:30 *WGs Meeting*

18:00 *Closing of the Sessions*
INVITED LECTURES
D1 protein is one of the rapidly turning over plant proteins with its half-life linked to light intensity. Along with the relatively more stable D2 protein, it forms the heterodimeric core of the photosystem II (PSII) reaction center. D1 is one of the most researched plastid proteins for over three decades, and yet the mechanisms and consequences of light-dependent D1 protein degradation are far from being fully understood [1]. Suggestions for light responsive roles for D1 turnover include: a compensatory mechanism for maintenance of a functional interaction of the proteins and lipids of the PSII complex [2]; PSII repair cycle to safeguard against photoinhibition [3]; a general adaptation signal for plant survival under drought [4] and anticipate changes in the environment [5]. Amino acid mutations (substitutions) in specific regions of D1 have now been linked to plant responses to ambient temperatures [6] and radiation pressure [7]. Some of these mutations affect D1 structural stability vis a vis other components of PSII complex [8]. Thus, these developments highlight the contribution of D1 conformation and turnover in stabilizing (or enhancing) PSII function in photosynthesis and oxygen production. In this lecture, I will summarize the salient features in the life history of the D1 protein. Then I will rewind the clock back to a speculation [9] that ‘…a signal whose amplitude varies with the rate of 32 kDa (nee D1) protein turnover acts as the plants’ light meter, setting into motion a process that helps the plant to adjust to changing light conditions. This adjustment could involve rapid reorganization of the lipid and protein components of the chloroplast for optimal photosynthetic efficiency by maintaining a functional interaction of the light harvesting chlorophyll a/b apoprotein, the regulatory protein components (such as the 32 kDa-D1 protein), and lipids of the PSII complex’. Finally, a model will be presented and discussed which will include evidence favoring our contention that D1 protein conformation change (and likely its turnover rate) functions as a ‘stimulus’ in the chloroplast which cross talks extra-plastidically to modulate specific cellular metabolism and help the plant to survive in extreme environments.

References

Acknowledgments
I wish to thank and acknowledge the contributions of my postdoctoral associates over the years, superb collaborators Marvin Edelman (Israel), Maria Teresa Giardi (Italy) and Giuseppina Rea (Italy) and their team members who all have significantly contributed to the investigations to be discussed in this lecture.
INVITED LECTURES

IN SILICO MODELLING OF PHOTOSYNTHETIC ELECTRON TRANSPORT
IMRE VASS, LÁSZLÓ SASS, ZSUZSANNA DEÁK
Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

We have developed a computer model of the complex network of electron transport components in the thylakoid membrane of photosynthetic organisms. The model provides an excellent tool to simulate electron transport processes in a wide range of conditions, and can be used to perform in silico experiments, whose predictions can be verified by measuring the kinetics of various electron transport components. The model was used to study the interpretation of transition probabilities in the water oxidizing complex. Our results show that the well-known period-four oscillation of oxygen evolution, as well as of the individual S-states can be described by the equilibrium of forward and backward electron transport reactions without the need for specific “miss” and “double-hit” events. We have also used our in silico photosynthesis model to interpret the recently described wave phenomenon in the relaxation of flash-induced Chl fluorescence in cyanobacteria and other microalgae. Our results show that the fluorescence wave reflects changes in the redox level of the PQ pool, which are caused by the imbalance of PSII and PSI electron transport and the feedback of electrons from stromal components to the PQ pool via the NDH-1 complex. This phenomenon provides an excellent tool to study the interaction of photosynthetic and metabolic electron transport and helps to identify the electron transport components, which are involved NDH-1 mediated cyclic electron flow. The model represents a systems biology approach to electron transport studies and has a large potential for optimizing electron transport pathways for biotech purposes.

References

Acknowledgement
This work was supported by the Hungarian granting agency OTKA (NN-110960).
PHOTOCATALYTIC H₂ EVOLUTION: FROM HOMOGENEOUS SYSTEMS TO PHOTOELECTRODE MATERIALS

V. ARTERO
Laboratoire de Chimie et Biologie des Métaux, Université Grenoble Alpes, CNRS, CEA, Grenoble, France.

Mimicking photosynthesis and producing solar fuels is an appealing way to store the huge amount of renewable energy from the sun in a durable and sustainable way. Hydrogen production through water splitting has been set as a primary target for artificial photosynthesis [1] which requires the development of efficient and stable catalytic systems, only based on earth abundant elements, for the reduction of protons from water to molecular hydrogen. We will report on our contribution to the development of various series of catalysts for H₂ evolution, namely from the cobalt diimine-dioxime series [2], and show how such molecular catalysts can be coupled with photosensitizers to prepare light driven molecular devices for H₂ evolution [3]. Such homogeneous systems use sacrificial electron donors and are therefore difficult to integrate in an overall water splitting system. We will finally report on our recent progresses towards the preparation of photoelectrode materials [4,5] that can be implemented into photoelectrochemical (PEC) cells for water splitting.

References

Acknowledgements
This work was supported by the French National Research Agency (Labex program, ARCANE, ANR-11-LABX-0003-01), the FCH Joint Undertaking (ArtipHyction Project, Grant Agreement n.303435) and the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement n.306398.
Neutrons are elementary particles whose de Broglie wavelengths of about 2 to 10 Å fall in the range of interatomic distances, while their energies of about 1 to 20 meV are in the order of low-energy dynamical excitations, like localized conformational protein motions, methyl group rotations or low-frequency protein vibrations. Therefore, neutron scattering techniques can be used for investigations of both structural and dynamical properties of photosynthetic pigment-protein complexes or membranes under close to in vivo conditions [1,2]. This presentation will give an overview about selected applications of different neutron scattering techniques in photosynthesis research.

First, we have used small-angle neutron scattering (SANS) to study the structure of light-harvesting complex II (LHC II) and Photosystem II (PS II) in buffer solution [3] at room temperature. Here, we made use of the fact that neutron scattering cross sections may vary significantly upon isotope exchange so that one may highlight scattering from specific sample parts by contrast variation. This allows to distinguish protein scattering from that of a detergent belt, a lipid bilayer and/or the solvent.

Furthermore, neutron diffraction and spectroscopy was employed to characterize the lamellar spacing and dynamics of hydration water layers of PS II membrane fragments. Hydration was also shown to be an important factor in functionalizing the PS II reaction center, because the \( Q_A \rightarrow Q_B \) electron transfer is active only above a hydration level of 45% relative humidity (see [1] and references therein).

Finally, neutron spectroscopy reveals vibrational and conformational dynamics of LHC II [4,5] up to physiological temperatures, while optical methods including single molecule spectroscopy are restricted to cryogenic temperatures. Our findings indicate that SANS, neutron diffraction and spectroscopy are valuable methods to characterize protein structure and dynamics at physiological temperatures.
ORAL PRESENTATIONS
INTEGRATED APPROACH TO CHARACTERISE THE FUNCTIONAL ARCHITECTURE OF PLANT PHOTOSYSTEMS

STEFANO CAFFARRI
Aix Marseille Université, CEA, CNRS, UMR 7265 Biologie Végétale et Microbiologie Environnementales, Lab. de Génétique et Biophysique des Plantes, 13009 Marseille, France

By absorbing light energy and working in series, Photosystem II (PSII) and Photosystem I (PSI) drive oxygenic photosynthesis by splitting $H_2O$ molecules and feeding an electron transport chain that ultimately reduces inorganic carbons into organic matter. Both photosystems are large membrane supercomplexes composed of several proteins and cofactors and structurally/functionally organized in two moieties: the core complex and the antenna system. The core complex, where photochemistry is performed, is well conserved amongst all oxygenic photosynthetic. Conversely, the antenna systems are very different, being composed by integral membrane complexes (Lhc) in eukaryotes and peripheral thylakoid-anchored complexes (phycobilisome) in cyanobacteria. The determination of the high resolution structure of photosystems is of paramount importance to understand their functioning. While the high resolution structures of both cyanobacterial photosystems have been solved thanks to the isolation of stable complexes from thermophilic bacteria [1, 2], in the case of eukaryotes, the high resolution structural model of PSII is far from being attained and the almost-complete plant PSI high resolution structure has been solved only very recently [3]. The main issue for the structural/functional characterization of the eukaryotic PSII and PSI supercomplexes is the isolation of stable and homogeneous complexes. The antenna system is indeed composed of integral Lhc membrane proteins (Light harvesting complex), which, having weak and different strengths of binding to the core complex, are partially lost during photosystem purification leading to heterogeneous and unstable preparations. Our investigation has been focused on the development of protocols to purify stable and homogeneous PSII-LHCII and PSI-LHCII supercomplexes from higher plants [4, 5]. Then, by using an approach integrating biochemistry, spectroscopy and electron microscopy, we have been able to reveal important aspects about the supramolecular organization, the composition and the functioning of plant photosystems. In particular, we have investigated the specific protein content of the LHCII complexes (the heterotrimeric main Light harvesting complex of PSII) occupying different positions in the supercomplex and we found that different Lhcb1-2-3 isoform composing LHCII have different role in the structure and regulation of photosystems. We also studied the energy transfer kinetics in PSII-LHCII and PSI-LHCII supercomplexes by time resolved fluorescent techniques and mathematical modeling of the data. Finally we have improved structural information about the plant PSII complex using a single particle electron microscopy approach.

References

Acknowledgement
SC is supported by the French National Research Agency Grant ANR-12-JSV8-0001-01.
Photosynthetic organisms evolved a natural capacity to modulate photosynthetic activity in response to varying light and other environmental conditions. In low light they need to harvest every available photon to sustain life, while in high light they dissipate the energy absorbed in excess to avoid photodamage. Light-Harvesting Complexes (LHCs) are pigment-protein systems responsible for photon absorption and transfer of the excitation energy to the reaction center, where charge separation occurs. It is generally believed that LHCs can change their function from a light harvesting to a photoprotective mode by switching between different conformations. However, the underlying molecular picture has not been elucidated yet. The available crystal structures represent the quenched form of the complex, while solubilized LHCII has the properties of the unquenched state. To determine the structural changes involved in the switch and to identify potential quenching sites, we have explored the structural dynamics of LHCII, by performing a series of microsecond Molecular Dynamics simulations. We show that LHCII in the membrane differs substantially from the crystal and has the signatures that were experimentally associated with the light-harvesting state.

Next we address the question what triggers the conformational change. A series of experiments in which we study the effect of putative triggers (e.g. protonation and zeaxanthin binding) on the conformation of the LHCs will be presented.

References
THE PLASTID TERMINAL OXIDASE PTOX AS A SAFETY VALVE UNDER EXCESS LIGHT
KATHLEEN FEILKE AND ANJA KRIEGER-LISZKAY
Institute for Integrative Biology of the Cell (I2BC), Commissariat à l’Energie Atomique et aux
Energies Alternatives (CEA) Saclay, Institut de Biologie et de Technologie de Saclay, Centre
National de la Recherche Scientifique (CNRS), Université Paris-Sud, 91191 Gif-sur-Yvette cedex,
France

When isolated thylakoid membranes or isolated photosystem preparations are used in a technical
device, it may be useful to add a safety valve in analogy to the PTOX present in the natural system.
This safety valve should only operate when the photosystem (or both photosystems in case of
thylakoids) becomes saturated. To design such a safety valve, a better understanding of the
enzymatic reaction and the regulation of PTOX are needed.
We have characterized PTOX using recombinant PTOX from rice fused to the maltose-binding
protein (MBP-OsPTOX). MBP-OsPTOX was expressed in E. coli and purified by affinity
chromatography. The purified enzyme is highly active[1]. Addition of MBP-OsPTOX to photosystem
II-enriched membrane fragments results in a functional system showing electron transport from
photosystem II to PTOX [2].
PTOX activity has to be regulated so that it does not compete with electron flow under normal
conditions but does function as a safety valve under excess light. Using tobacco expressing
PTOX1 from C. reinhardtii [3], we demonstrate by thermoluminescence studies that PTOX is not
active in dark-adapted leaves while it is activated under steady state light conditions. We show that
PTOX is inactive when the pH in the stroma is neutral or only slightly alkaline. We propose a model
stating that the association of PTOX to the membrane is controlled by the transthylakoidal proton
gradient. According to this model PTOX has only access to its substrate plastoquinol when
photosynthetic electron transport is saturated.

References
plastid terminal oxidase from rice (Oryza sativa). Biochim. Biophys. Acta 1837, 1284–1292;

Acknowledgements
We thank P. Nixon (Imperial College London) for providing us with Cr-PTOX1 seeds. This work was supported
by the CNRS, the CEA and the Université Paris-Sud.
CHARACTERIZING TERMINAL OXIDASE AND FLAVODIIRON ELECTRON SINK FUNCTION IN THE CYANOBACTERIUM *SYNECHOCYSTIS* SP. PCC 6803

MARIA ERMAKOVA¹, TUOMAS HUOKKO¹, PIERRE RICHAUD², LUCA BERSANINI¹, DAVID J. LEA-SMITH³, CHRISTOPHER J. HOWE³, GILLES PELTIER², YAGUT ALLAHVERDIYEVA¹

¹Laboratory of Molecular Plant Biology, Department of Biochemistry, University of Turku, Finland;
²CEA, IBEB, Laboratoire de Bioénergétique et Biotechnologie des Bactéries et Microalgues, Cadarache, France; ³Department of Biochemistry, University of Cambridge, UK.

Various O₂ utilizing electron sinks, including the soluble flavodiiron 1/3 complex (Flv1/3) [1,2], and the membrane localized respiratory terminal oxidases (RTOs) [3,4], cytochrome c oxidase (Cox) and quinol oxidase (Cyd), are present in the photosynthetic electron transfer chain of *Synechocystis* sp. PCC 6803. However, the relative importance and functional role of these electron sinks under various environmental conditions is poorly understand. Via membrane inlet mass spectrometry gas-exchange, chlorophyll a fluorescence, P700 analysis and inhibitor treatment of wild-type and various electron sink mutants, we investigated the contribution of the three complexes to alleviation of excess electrons under light, dark and fluctuating light conditions. We demonstrate that Cyd is able to oxidise plastoquinol, although activity was detected only upon inhibition of electron transfer at the Cytochrome b₆f site and in ∆Flv1/3 under fluctuating light conditions, where linear electron transfer was drastically inhibited due to impaired PS I activity [5]. Cox is mostly responsible for dark respiration and competes with P700 for electrons under light. RTOs are able to partially substitute for each other and consequently only ∆cox/cyd demonstrated a highly reduced plastoquinone pool in darkness, and impaired gross O₂ evolution under light. Moreover, we provide clear evidence of an alternate electron transfer route bypassing Cyt b₆f and terminating at Cox. Our data demonstrates that all three electron sinks contribute to alleviation of excess electrons under illumination: RTOs continue to function under light operating on slower time ranges and on limited scale, whereas Flv1/3 responds rapidly as a light induced component and has greater capacity.

References


Acknowledgments

This work was financially supported by the Kone Foundation and the Academy of Finland Finnish Centre of Excellence in Molecular Biology of Primary Producers (2014-2019) project # 271832.
The in-depth understanding of the molecular mechanisms regulating photosynthetic processes in plants, algae and cyanobacteria may serve as inspiration for the design of biomimetic artificial systems that can be used to store solar energy in an environmentally friendly way. [1] One of the key issues for the success of such artificial photosynthesis material is the capability to perform water splitting into molecular oxygen and hydrogen equivalents. To achieve this challenging task photosynthetic organisms use a protein complex that remained almost unchanged during the evolution in the last two and a half billion years: the Oxygen Evolving Complex of Photosystem II. In such complex, the water splitting reaction proceeds by the accumulation of four oxidizing equivalents on the Mn$_4$CaO$_5$ cluster through five (S$_0$ - S$_4$) following states known as Kok’s cycle. The unravelling of the details of the water-splitting reaction mechanism of both the natural system and its synthetic analogues are of great relevance for understanding of natural photosynthesis and the design of efficient inorganic catalysts that may serve as artificial leaves. Based on the recently reported X-ray structure of the Photosystem II complex, we have deeply investigated the reaction details by means of DFT+U Quantum Mechanics / Molecular Mechanics calculations, pinpointing the role of two distinct geometrical structures of the Mn$_4$CaO$_5$ catalytic centre observed in the S$_2$ state. [2] On the grounds of our simulations we provide an interpretation of the temperature, illumination, and protocol dependence of historical EPR experimental data, which represented a puzzle for the scientific community since the 90s. [3a] The dynamics also suggest pathways for the movements of electrons, protons and substrate water molecules along the transition between the S$_2$ and S$_3$ states [3b,3c]. On the inorganic side, we firstly investigate cluster models of a cobalt-based amorphous catalyst (CoCat) in explicit water solution, providing insight into the pathways for oxygen evolution. We identified the formation of Co(IV)-oxyl species as the driving ingredient for the activation of the catalytic mechanism, followed by their geminal coupling with O atoms coordinated by the same Co. Concurrent nucleophilic attack of water molecules coming directly from the water solution is discouraged by high activation barriers [4]. Amorphous Manganese oxides for electrochemical water splitting have been also proposed as promising materials for large-scale generation of chemical fuel. We have recently explored the structural motifs of a manganese-based (MnCats) catalysts for oxygen evolution by combining the information content of X-ray absorption fine structure (XAFS) measurements with the predictive power of ab initio calculations. On the grounds of our results we are able to characterise the essential structural and electronic properties of MnCats, identifying in particular: (i) the localization and structural connection of Mn(II), Mn(III), and Mn(IV) ions in such amorphous oxides and (ii) the distribution of protons at the MnCat/water interface [5]. The achieved results suggest also interesting differences and similarities between the bio-inspired CoCat and MnCat, and the catalytic core of Photosystem II.

References
ORAL PRESENTATIONS

BIOINFORMATICS APPROACHES TO PHOTOSYSTEM II STRUCTURE, FUNCTION AND ENGINEERING
GIUSEPPINA REA¹, MAYA LAMBREVA¹, VIVIANA SCOGNAMIGLIO¹, AMINA ANTONACCI¹, PASQUALE STANO², VERANIKA ZOBNINA², MARIA TERESA GIARDI¹, GAETANO CAMPI¹, IVO BERTALAN³, UDO JOHANNINGMEIER³, FABIO POLTICELLI²
¹Institute of Crystallography, CNR, Rome, Italy
²Department of Sciences, Roma Tre University, Rome, Italy
³Plant Physiology Institute, Martin-Luther-University, Halle (Saale), Germany

Advances in computing power and refinement of computational biology tools has recently allowed to analyze increasingly complex systems through bioinformatics approaches. Photosystem II (PSII), a 350 kDa proteins cofactors macromolecular complex located in the thylacoid membranes of oxygenic photosynthetic organisms, is a prototype of such complex systems. PSII core is made up by the D1 e D2 proteins (the “reaction center”) which contain binding pockets for the plastoquinones QB and QA. PSII Catalyzes the light-induced production of reducing equivalents in the form of plastoquinol molecules. The reaction byproduct is molecular oxygen and thus PSII is essential for higher organisms life on Earth. Here, recent bioinformatics studies of PSII will be discussed focusing on the reaction center evolution and on the macromolecular complex dynamics and mechanism of plastoquinol-plastoquinone exchange. In addition, bioinformatics-driven engineering studies of PSII for biotechnological purposes will also be reported. Finally, the design and characterization of PSII mimics for biosensing purposes will be illustrated as well.

References

Acknowledgements
Support from the COST Action TD1102 “Photosynthetic proteins for technological applications: biosensors and biochips” (PHOTOTEC) is gratefully acknowledged.
We studied photosynthetic reactions by static and time-resolved differential FTIR in different systems. First, we studied the photoinduced electron transfer (ET) reactions and associated processes in bacterial reaction centers (RCs). Proton transfer, internal water molecule displacement, conformational changes were investigated under different conditions (series of flashes, continuous illumination, isolated RCs, membrane-bound RCs). The double reduction of the ubiquinone \( Q_b \) was followed in its different steps [1-3]. A chemometrical approach based on multivariate curve resolution made it possible to disentangle the different kinetic contributions [2-3].

In addition, we studied the effect the hydration of the RC, using an isopiestic method to operate under a given relative humidity (RH). We found that in a RC placed in a compartment with low relative humidity (RH = 11%) the ET reaction are strongly inhibited and the protein response to charge separation is very different from more hydrated samples [4,5]. More in details, dehydration influences:

1) the recombination kinetics of the light-induced charge separation between the primary electron donor (P) and the quinone acceptor (\( Q_A \)), both after a flash and after continuous illumination [4];
2) the displacement of internal water molecules associated with the \( Q_A/Q_A^+ \) transition [5];
3) the degree of charge delocalization in the primary donor [6];
4) In addition, strong changes are observed from NH or OH stretching modes of amino acid residues (3550–3150 cm\(^{-1}\) range) suggesting a modification of the internal rearrangements which stabilize the charge-separated state [5].

As a second system, we studied the photoprotective mechanism of diatoms in vivo. In this case in was possible, on a second time scale, to follow by rapid-scan FTIR the response of the microorganism to strong light, implying a xanthophyll cycle. We could follow different events (chemical epoxidation/de-epoxidation of carotenoids; plastoquinone pool reduction; localized pH changes; energy dissipation mechanism) in real time. The obtained data were correlated with results from Resonance Raman, chromatography, and fluorescence to obtain a global picture of the whole biochemical process [7].
MODELING OF P*H_A^- CHARGE RECOMBINATION IN RHODOBACTER SPHAEROIDES REACTION CENTERS ON THE BASIS OF BOTH ABSORPTION AND FLUORESCENCE TIME-RESOLVED STUDIES

KATARZYNA DUBAS¹, MICHAŁ BARANOWSKI², ARTUR PODHORODECKI², MIKE JONES³, KRZYSZTOF GIBASIEWICZ¹

¹Adam Mickiewicz University in Poznań, Department of Physics, ul. Umultowska 85, 61-614, Poznań, Poland
²Institute of Physics, Wrocław University of Technology Wybrzeże Wyspianskiego 27, 50-370 Wrocław, Poland
³School of Biochemistry, Medical Sciences Building, University of Bristol, University Walk, Bristol, BS8 1TD, UK

In order to successfully apply photosynthetic reaction centers (RC) in photovoltaic devices, a basic knowledge on their functioning is necessary. Reaction center from purple bacterium Rhodobacter sphaeroides is one of the best characterized [1]. Still, however initial steps of electron transfer in this system, critical in photovoltaic applications, are not fully understood. Particularly, the role of protein dynamics – a factor which makes a difference when comparing this system to purely artificial light sensitive materials – needs to be further explored.

Involvement of protein dynamics in electron transfer inside the Rba. sphaeroides RC was proposed more than 30 years ago on the basis of multiexponential fluorescence decay originating from P*H_A^- charge recombination leading to delayed fluorescence from P*, where P and H_A are the primary electron donor and acceptor, respectively [2]. Observation of three exponential lifetimes of ~0.7, ~3 and ~10 ns was explained by increasing free energy gap between P* and P*H_A^- due to protein reorganization in response to the appearance of the charges on P and H_A^-.

However it is well known, that there is another intermediate state, P*B_A, formed both during the forward and backward electron transfer between P(+)(+)+ and H_A^(-)(-), B_A being an intermediate electron carrier located halfway between P and H_A^-.

Till recently it was commonly accepted, that during P*H_A^- charge recombination, transient absorption signal, unlike fluorescence decay, was monoexponential. This situation caused some interpretational difficulties. Recently, however, it was documented that charge recombination in RCs closed by prereduction of the secondary electron acceptor Q_A leads to multieponential decay also of transient absorption signals [3]. This situation urged us to develop a kinetic-energetic model of primary electron transfer events combining the results from both techniques. As a result we were able to propose temporal evolution of the free energy level of the states P*H_A^- and P*B_A relative to P*. Surprisingly, we found that whereas the free energy gap between P* and P*B_A increases with time, a gap between P* and P*H_A^- is getting smaller in response to protein reorganization. This unexpected result may be elegantly explained by gradual polarization of the protein environment, which stabilizes the state P*H_A^- and destabilizes the state P*B_A.  

References

Acknowledgements
K.G. acknowledges financial support from the National Science Center, Poland (project entitled "Biosemiconductor hybrids for photovoltaic cells" no. 2012/07/B/NZ1/02639.)
DETECTION OF HERBICIDE USING CHLOROPLAST-HERBICIDE INTERACTION BY RAMAN SPECTROSCOPY

ISMAIL HAKKI BOYACI1,2, H. SEBNEM ACIKBAS1, ESRA ACAR-SOYKUT2, OZAY MENTES2
1Hacettepe University, Department of Food Engineering, Beytepe, 06800, Ankara, Turkey
2Hacettepe University, Food Research Center, Beytepe, 06800, Ankara, Turkey

Depending on population growth, demand for food requirement is increasing. Thus high food crops which are undamaged are needed. For this reason, it is necessary that foods must be protected against some animal, plant and microorganism. Herbicides are used to tackle these harmful weeds. However, unconscious and overuse of these herbicides, not only damages the plants but also damages all the living creatures and the nature. All of the known herbicides used in agriculture, function in the inhibition of as photosynthesis means of different actions (such as inhibition of amino acids, inhibition of lipid synthesis, growth disorder and cell membrane disruption) via binding to the secondary Quinone (Qb) site as a target molecule [1]. The binding affinity of chemicals depends on the structural conformation of the quinone-binding site Qb where they are found in chloroplasts that is center content of chlorophyll. Nowadays, photosynthetic materials are used in different technological studies. Of these, biosensors for detection of environmental pollutants, photovoltaic devices for power generating and photosensors, phototransistors and fuel cell [2,3]. Among these applications, the most popular are biosensors, which are used for detection of herbicides [4]. Consolidated techniques for the detection of herbicides are high performance liquid chromatography and gas chromatography coupled to mass spectrometry. Although very selective, these techniques do not provide fast detection. In addition, data processing becomes very complex in the presence of samples containing different herbicides. Moreover operation of these instruments requires trained personnel and expensive instrumentation in a professional laboratory environment [5]. There is need for a rapid and sensitive novel method for detection of herbicides such as Raman Spectroscopy analysis. In this study, a rapid and reliable method was developed for the determination of herbicide using Raman spectroscopy. For this purpose, chloroplast was isolated from spinach (Spinacia oleracea) and the interaction of chloroplast-herbicide was monitored using dispersive Raman spectroscopy. A linear correlation was obtained between the herbicide concentration and Raman intensity in the range of 12-1360 ppm with a coefficient of determination of 0.972. The limit of detection for homogeneous assay was determined as 5.2 ppm. Validation of the new method was performed. The new method has been successfully applied to real samples and the results were compared with LC-MS/MS results. The results showed that this method will be a potential tool for the determination of herbicide using Raman spectroscopy.

References
DETECTION OF CAROTENOID BIOSYNTHESIS, PPO AND ALS INHIBITING HERBICIDES USING RAMAN SPECTROSCOPY AND CHLOROPHYLL FLUORESCENCE

KAREL KLEM, PETR VÍTEK
Global Change Research Centre AS CR, v.v.i.

Raman spectroscopy and chlorophyll fluorescence represent both non-invasive, optical methods with high potential for applications in biosensor technologies for detection of herbicide residues. Within this study we analysed the changes in Raman spectra excited by 785nm laser and chlorophyll fluorescence kinetics in Galium aparine and Helianthus annuus leaves treated by herbicides belonging to groups of carotenoid biosynthesis, PPO and ALS inhibitors. The ratios of Raman bands for chlorophyll (1550 cm\(^{-1}\))/carotenoids (1525cm\(^{-1}\)) or phenolics (1605 cm\(^{-1}\))/carotenoids(1525cm\(^{-1}\)) showed most pronounced response to herbicides and can be considered as good indicator of these inhibitors in plant. Potential for mapping the spatial distribution of herbicide within the leaf using Raman spectroscopy has also been demonstrated. Chlorophyll fluorescence imaging revealed also considerable potential for detecting selected groups of herbicides, wherein range of parameters derived from both slow and fast kinetics of fluorescence was identified. Using both methods for detection of herbicide residues in environment represents therefore a complementary tool, particularly if the herbicide mode-of-action is unknown.

Figure 1. A) Raman map based on carotenoid (v\(_{1}\) C=C signal to baseline intensity) spatial distribution showing herbicide-induced carotenoid depletion within sunflower leaf (Helianthus annuus) after application of herbicide diflufenican. B) Response of chlorophyll fluorescence parameter V\(_{j}\) to application of full and half dose of three types of carotenoid biosynthesis inhibitors (mesotrione, clomazone and diflufenican) and one ALS inhibitor (amidosulfuron) in sunflower leaves.

Acknowledgments
This work was supported by Ministry of Agriculture of Czech Republic, project no. QJ1530373.
PHOTOSYNTHESIS MONITORING TO ESTIMATE GROWTH IN MICROALGAE CULTURES: CHLOROPHYLL FLUORESCENCE TECHNIQUES

JIŘÍ MASOJÍDEK¹,², JOSÉ R. F. MALAPASCUA¹,², KAROLINA RANGLOVÁ¹, MAGDA SERGEJEVOVÁ¹

¹Laboratory of Algal Biotechnology, Institute of Microbiology, Academy of Science, Opatovický mlýn, Třeboň, Czech Republic;
²Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Microalgae (phototrophic microorganisms including photosynthetic bacteria, prokaryotic cyanobacteria and eukaryotic algae) have been cultivated commercially in mass cultures as food and feed additives, for pharmacology and cosmetics, for waste water treatment and biotests, and most recently as a potential source of biofuels [1, 2]. In their biomass, microalgae produce various bioactive substances such as pigments, polyunsaturated fatty acids, antioxidants, or immunologically-effective compounds. Growth of outdoor microalgae cultures is influenced by changes of photosynthetic performance that depends on environmental conditions, particularly light and temperature as well as other physico-chemical variables, such as CO₂/O₂ exchange, nutrients, turbulence, etc [3]. In microalgae biotechnology we make an effort to optimise the culture growth, namely to use as much light energy for photosynthetic production as possible. Since the mid-1990s chlorophyll (Chl) fluorescence has been used to monitor photosynthetic performance of microalgae mass cultures and optimise their growth [4] due to its non-invasiveness, rapidness, ease-to-use, sensitivity as well as wide availability of reliable instruments and advanced theory of data interpretation. We have aimed to set-up simple procedures and guides for physiologists and biotechnologists on how Chl fluorescence can be used to monitor changes in photosynthetic activity of microalgae cultures and how to correlate them to the growth and productivity. Here, we demonstrate the two most common fluorescence techniques fast fluorescence induction kinetics (OJIP-test) and pulse-amplitude-modulation analysis of fluorescence quenching. The emphasis was given on the applications and limitations of Chl fluorescence techniques through several case studies. Chl fluorescence characteristics of various microalgae cultures were studied on-line/in-situ in laboratory and outdoor cultivations. Changes in selected variables monitored by these techniques can be related to changes of cultivation conditions, physiology, and growth of microalgae cultures for a given strain and cultivation system. By data analysis of culture samples we can evaluate photosynthetic activity as electron transport rate, photosynthetic efficiency, irradiance saturating photosynthesis as well as reduction status of the Photosystem II complex, the distribution of absorbed energy between photochemistry and dissipative pathways. This set of fluorescence variables provides a clear and complex picture of microalgae photosynthetic performance with higher resolution as compared to, for example, spectroscopy or oxygen production measurements. In conclusion, Chl fluorescence measurements can be used in both indoor and outdoor studies for strain selection and characterisation, monitoring physiological status, optimisation of cultivation procedure in various systems. In this way, we can also correlate photosynthetic activity with growth and biomass productivity and cultivation process may be controlled.

References

Acknowledgements
This research was supported by the ministry of education, youth and sports of the czech republic, project algatech cz.1.05/2.1.00/03.0110, algain cz.1.07/2.3.00/30.0059 and algatech plus lo1416.
ORAL PRESENTATIONS

PHOTOSYNTHETIC PROTEINS FOR LIGHT ENERGY CONVERSION INTO ELECTRICITY AND SOLAR FUELS
NICOlas Pluméré1, FangyuAn zhao2, volKer Hartmann3, felipe Conzuelo2, Marc M. NowsAczyK1, Matthias Rögner3, wolfgang SchuHMann2
1Center for Electrochemical Sciences, Ruhr-Universität Bochum, Bochum, Germany.
2Analytical Chemistry, Ruhr-Universität Bochum, Bochum, Germany.
3Plant Biochemistry, Ruhr-Universität Bochum, Bochum, Germany.

Natural photosynthesis converts solar light into biomass. The photosynthetic proteins involved in light harvesting and charge separation can be integrated in artificial devices to directly produce electricity and chemical fuels. We exploit redox hydrogels to immobilize and electronically contact the photosynthetic protein to electrodes. The redox potentials of the electron relays and the properties of the polymeric supporting matrix are tuned to enable maximal current densities at low overpotential. To illustrate the desired parameters of the redox hydrogels, the example of Z-scheme inspired biophotovoltaic cells will be given [1, 2]. In particular, the performances of a photosystem 1 based photocathode are sufficiently high to envision technological applications in biophotovoltaic devices [3]. Besides the solar energy to electricity conversion, light induced H₂ evolution from a bio-photocathode based on photosystem 1 - platinum nanoparticles complexes integrated in a redox hydrogel film will be presented [4].

References

Acknowledgments
Financial support by the COST action TD1102 (PHOTOTECH) and the Cluster of Excellence RESOLV (EXC 1069) funded by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.
PLASMONIC ENHANCEMENT OF PHOTOCURRENT GENERATION AND STABILITY OF BIO-HYBRID NANOSTRUCTURED PIGMENT-PROTEIN SILVER ELECTRODES

VINCENT M. FRIEBE¹, JUAN D. DELGADO¹, DAVID J. K. SWAINSbury², J. MICHAEL GRUBER¹, ALINA CHANAewA¹, RIENK VAN GRONDELLE¹, ELIZABETH L. VON HAUFF¹, DIEGO MILLO¹, MICHAEL R. JONES², RAOUl N. FReSE³

¹Department of Physics and Astronomy, LaserLaB Amsterdam, VU University Amsterdam, De Boelelaan 1081, Amsterdam 1081 HV, The Netherlands
²School of Biochemistry, Medical Sciences Building, University of Bristol, University Walk, Bristol BS8 1TD, United Kingdom.

In a quest to fabricate novel solar energy materials, the high quantum efficiency of photosynthetic pigment-proteins is being exploited through their direct incorporation or mimicry in bioelectronic devices. Here we examine photocurrent generation by bacterial reaction centre-light harvesting 1 (RC-LH1) complexes self-assembled on a nanostructured rough silver substrate. This novel bio-hybrid electrode yielded a peak photocurrent of 166 µA cm⁻² under 1 sun illumination, and a maximum of over 400 µA cm⁻² under 4 suns. We measured a 2.5-fold increase in light absorption per RC-LH1 complex, due to plasmonic enhancements from the substrate. We confirmed these plasmonic interactions using confocal fluorescence microscopy, revealing an increase of fluorescence yield and radiative rate of the RC-LH1 complexes. Nano-structuring of the silver substrate also enhanced the stability of the protein under continuous illumination by almost an order of magnitude relative to a non-structured bulk silver control. Due to its ease of construction, increased protein loading capacity, enhanced stability and more efficient use of light, this hybrid material is an excellent candidate for further development of plasmonically-enhanced biosensors and bio-photovoltaic devices.
Metal oxide semiconductors are used in photocatalysis to destroy organic toxicants. The same semiconductors are used in photoelectrochemistry as support matrix for organic dyes and sensitizers, which adds to their functionality, for instance in solar cells. This apparent paradox around two fields of technology deserves further investigation.

We deposited the light harvesting pigment-protein complex phycocyanin on iron oxide photoanodes and experienced increased photocurrents in photoelectrochemical water splitting reactors [1]. Such bio-hybrid electrodes may have advantages over all-inorganic devices, but a lot of open questions exist, particularly that about the charge transfer between the light antenna proteins and the substrate [2]. The question becomes even more important when complete algal biofilms are grown on such electrodes. We show how we synthesize and process such bio-hybrid electrodes and anchor them on solid substrates and operate them in photoelectrochemical cells for solar fuel production. The charge transfer of physisorbed, chemisorbed light antenna with genetically engineered linkages [3] and complete biofilms is assessed with electroanalytical methods (impedance spectroscopy) [4] and with synchrotron based resonant valence band spectroscopy [5]. We show how we step-by-step zoom in into the complexity of the bio-electric interface and photosynthetic apparatus with increasingly complex instrument arrangements which we operate in situ and operando under realistic bio-electrophysiological and photosynthesis condition.

References

Acknowledgments
Swiss Federal Office of Energy (BaselPEC); Sciex 10.013, VELUX Foundation # 790, COST TD1102, SBFI C13.0083.
Recent research in spintronics is aimed at introducing spin-based devices in medical and biological applications. An essential component in these devices is the spin injector. We observed very high spin selectivity in electron transfer (ET) in photosystem I (PSI). Light-induced spin injection was observed by adsorbing the PSI complex on a silver substrate, exciting it with red light and using a spin analyzer device developed recently for detecting spin polarization. We found that the photoexcitation-induced charge separation across PSI is spin selective and that the system has an efficiency of almost unity in spin-specific injection. Temperature dependence studies revealed that the injection is especially efficient at room temperature. The observation that the nano-size protein operates at room temperature and in a dry environment renders PSI as a possible spintronic component for applications in nano optoelectronic devices. At physiological relevant temperatures the spin selectivity is extremely high and is superior to the spin polarization obtained by most inorganic spin injectors at room temperature. This finding opens the possibility of combining spintronics with bio-applications having a bio-system-based spin injector. Since the system properties are not related to the exchange interaction, that controls magnetism, this system can be miniaturized to a scale of a few nm. This size cannot be obtained with ferromagnetic-based devices which become superparamagnetic and their spin orientation is not stable at temperatures relevant to many applications.
SYNTHESIS OF A NEW 1,8-NAPHTHALIMIDE BASED PAMAM-TYPE DENDRON AND INVESTIGATING ITS POTENTIALITY FOR ARTIFICIAL LIGHT-HARVESTING

IVO GRABCHEV¹, STANISLAVA YORDANOVA², STANIMIR STOYANOV², IVAN PETKOV²
¹Sofia University “St. Kliment Ohridski”, Faculty of medicine, 1407 Sofia, Bulgaria,
²Sofia University “St. Kliment Ohridski”, Faculty of Chemistry and Pharmacy, 1153 Sofia, Bulgaria.

The artificial light harvesting system, which absorbs light and transfer energy to the reaction center, have a very important role in the scientific researches. Many artificial light-harvesting molecules are synthesized in succession. In such systems the excitonic energy resulting from light absorption of chromophores is efficiently funnelled to the other chromophore in the same system. The latter behaviour is termed antenna effect. An effective antenna dendron comprising two differently substituted 1,8-naphthalimide fluorophore units has been synthesized for the first time implementing relatively simple procedure (Scheme 1). The photophysical characteristics of the compound have determined in solvents of different polarity. The values for the quantum fluorescent yield obtained in polar values are lower than those registered in non-polar solvents. This fact demonstrates the medium dependent properties of the newly synthesized molecule. The 98% energy transfer from the donor 4-ethoxy-1,8-naphthalimide units to the acceptor 4-aminosubstituted-1,8-naphthalimide unit observed during the investigations in all organic solvents evidences the antenna capacities of the dendron. This opens opportunities to use this simple dendron as an effective artificial energy transfer system.

Scheme 1. Proposed mechanism of energy transfer of Dendron-

Acknowledgments
The authors would like to thank of COST TD1102.
COVALENT ATTACHMENT OF PHOTOSYNTHETIC REACTION CENTERS TO HYDROGEN-BONDED ORGANIC SEMICONDUCTORS FOR BIO-OPTOELECTRONIC APPLICATIONS.
F. MILANO¹, A. ANTONUCCI², R. R. TANGORRA², H. COSKUN³, D. FARKA³, Y. KANBUR³, A. OPERAMOLLA², E. D. GLOWACKI³, S. SARICIFTCI³, M. TROTTA¹, G. M. FARINOLA²
¹CNR-IPCF UOS Bari, Via Orabona 4, 70125 - Bari, Italy.
²Università degli Studi di Bari Aldo Moro, Dipartimento di Chimica, Via Orabona 4, 70125 - Bari, Italy.
³Linz Institute for Organic Solar Cells (LIOS), Physical Chemistry, Johannes Kepler University, Linz (Austria).

Hydrogen-bonded pigments are a class of organic colorants, which features many natural-origin molecules that have been used for centuries, as well as numerous mass-produced industrial synthetic compounds used in applications as various as out-door paints, cosmetics, and printing inks. Their widespread use in the dye and pigment industry is motivated by three favorable properties: low-cost production, excellent stability, and low toxicity, with some of them considered less hazardous than even water-soluble food dyes. Recently, H-bonded pigments have emerged also as promising organic semiconductors, with epindolidione (EPI) and quinacridone (QNC) (left panel in the figure) demonstrating hole mobility in the range of 0.1–1 cm² V⁻¹ s⁻¹ and outstanding operational stability in both air and in aqueous environments with pH 3–10 [1]. This latter finding is motivating for deploying these materials in applications requiring direct interfacing with biological “wet” environments. Their N–H and C=O functional groups are the “chemical handles” that are in principle amenable for direct bioconjugation.

A proper combination of the photosynthetic reaction center (RC), the pivotal protein in photosynthesis, with engineered materials such as metals or inorganic semiconductor electrodes, has attracted great attention for the building of new versatile hybrid devices for solar energy conversion [2]. Here we propose a covalent approach able to stably anchor RCs onto evaporated thin films of EPI and QNC. The N–H functional group of these molecules in thin film reacts spontaneously with N-hydroxysuccinimide functionalized linkers as disuccinimidyl suberate. The protruding linkers are then used to covalently bind the lysines residues of the Rb. sphaeroides RC, by forming an amide linkage (right panel in the figure). Our protocol is shown to preserve the semiconducting properties of the pigments while maintaining the protein’s photoactivity. Multiple reflection IR spectroscopy and AFM demonstrated the effective covalent binding and the robustness of the protein anchoring even after buffer washing. Furthermore, RC charge recombination kinetic measurements confirmed the full functionality of bioconjugated proteins ruling out any possible hindering effect from the organic films. As key results of our work, we show that semiconductors preserve their favorable electrical properties and the proposed photoconductor device operates under water, before and after RCs anchoring. These are enabling steps for using H-bonded pigments as a platform for multifunctional bioelectronics devices.

References
FROM WHOLE CELLS TOWARDS PHOTOSYNTHETIC REACTION CENTRES: “FUNCTIONAL” AND INTRINSIC DYNAMIC PROPERTIES

DANIELA RUSSO1,2, MAYA LAMBREVA3, GAETANO CAMPI3, GIUSEPPINA REA3

1 CNR Istituto Officina Materiali, c/o Institut Laue Langevin, 6 rue J. Horowitz BP156, F-38042 Grenoble, France
2 Institut Lumière Matière, UMR 5306 Universite Lyon 1 et CNRS
3 CNR Istituto di Cristallografia 00015 Monterotondo Scalo, Rome, Italy

The core of the reaction center protein is dominated by the D1/D2 heterodimer hosting all the redox cofactor involved in charge separation and electron transfer processes. The D1 protein is the subject of intense research being either the main actor in Photosystem II (PSII) assembly and repair cycle. Several studies demonstrated that even single point mutations in the D1 primary structure could affect PSII photochemistry and the physiological performance of the hosting organisms.

Here, we address the question if there is a “functional” dynamics in addition to the intrinsic dynamical behaviour common to all proteins and how do they couple. In particular, understanding if “rigidity” is essential for the charge transfer process and if this property is shared by all the photosynthetic systems and how this information can be apply to design high performant biosensors.

To this end a comparison between Chlamydomonas cells carrying both native and mutated D1 protein (hosted in the PSII of the cell) has been undertaken using neutron scattering experiment. Mutation were located in the functionally important regions D1 protein. All the mutants had a lower chlorophyll content indicating a possible modified antenna size. However, the mutation’s type and localization impacted photosynthetic performance in a different manner. Mutants displayed reduced electron transport efficiency in physiological conditions, and increased photosynthetic performance stability and oxygen evolution capacity in stressful high-light conditions.

Results show that point genetic mutations may notably affect not only the biochemical properties but also the T dependence of the whole complex dynamics in particular suggesting the wild type more rigid than mutants. We highlight non negligible differences at longer time scale, rather than short, and large scale. We defined an intrinsic soft matter dynamics and a “functional dynamics”. We bring to light for the first time that hydration water collective density fluctuations can provide also a measurement of “functional dynamics”. Finally, delay and prompt fluorescence results also suggest a different behavior between mutants and wild type samples.
PHOTOSYNTHETIC BASED-BIOSENSOR AND APPLICATIONS OF NEW STRATEGIES FROM BASIC RESEARCH TO MARKET

MARIA TERESA GIARDI¹,²
¹ Institute of Crystallography, Area Research of Rome, CNR, Rome, Italy
² Biosensor s.r.l, Rome, Italy

Concerning biosensors based on photosynthesis, the integration of different technologies allows the design and development of photosynthetic microorganisms engineered and synthetic peptides, which mimic parts of the photosynthetic apparatus, with a higher performance that can be used as elements of bio-recognition for the detection of environmental contaminants. Thus, the combination of computational analysis, molecular biology and tools biomimicking makes possible the realization of biomediators more stable, sensitive, selective and specific for the creation of effective biosensors. The improvement of these parameters is critical for reliability biosensor, and attract strongly the interest of commercial companies in an effort to accelerate the acceptance of this technology. Nowadays, genetic engineering allows you to edit specific nucleotide sequences of a genome body to get protein with improved properties and new innovative biotechnological approaches to help integrate these systems or their functional substructures, in manufactured assemblies for specific applications. The activities were performed with various types of photosynthetic microalgae like Chlamydomonas and Chlorellae. One of the biggest efforts was to make the photosynthetic material resistant the oxygen singlet and ROS, getting a long half-life of months, necessary to implement the biosensors.

The biosensor is drawn, as composed by the biomediators, the transducers (optical/amperometry/impedance) and the microfluidics. It is essential defining the right modality of interface between biological materials and the other biosensor components, as definition of the wavelengths of excitation-emission and best conditions of the biomediator state (e.g light or dark condition for whole cells) and operating state (free or immobilized).

Fundamental is also the application of new technologies based on quantum dots, nanoparticles and magnetic particles leading to an improvement in the response of photosynthetic material allowing easy transportation of the electronic signal.

We report our efforts from basic research to commercialization of lab-on-a chip photosynthetic biosensor.

References

Acknowledgments
This work is supported by Photosynchlamy ESA project, Bravoo EU Call for Ocean of tomorrow and Cost Action Phototech.
The *Rhodobacter sphaeroides* photosynthetic reaction center is a robust and malleable integral membrane protein that lends itself to the construction of biohybrid devices for a range of applications in photovoltaics, biosensing, photocatalysis, molecular electronics and synthetic biology. One key issue in the use of such proteins is controlling how they interact with conducting surfaces and other device components in order to optimise function, maximise stability and simplify fabrication procedures. To date these issues have largely been addressed through selection and surface modification of electrode materials, and the manipulation of mediators and reaction conditions. However, engineering of the proteins themselves to control adsorption and assembly at the electrode surface through genetic encoding remains largely unexplored. To this end we have modified the atomic structure of the reaction center protein in variety of ways to direct specific associations with conducting materials and to gain control over the way that individual proteins self-assemble into long-range oligomeric structures both *in vivo* and *in vitro*. The outcomes of this protein engineering will be discussed.

**Acknowledgements**

The authors acknowledge funding from the Biotechnology and Biological Sciences Research Council of the United Kingdom.
Photosynthetic reaction center protein (RC) purified from Rhodobacter sphaeroides R-26 was immobilized to porous silicon microcavity (PSiMc) by two methods. First method involves the deposition of a specific hydrophobic peptide layer (SPGLSLVSHMQT) as a strong physical interface between the RC and PSiMc. This new type of hybrid material showed considerable photoactivity when measuring the photocurrent after excitation by light in a special electrochemical cell. With the other method mitochondrial cytochrome c, an in vitro electron donor similar to the in vivo donor to the RC (cytochrome c2) was attached chemically to the PSiMc after silanization, and the RC was electrostatically bound through its docking site. The specific binding of RC was verified by monitoring the shift in the reflectance spectrum of the PSiMc. Flash photolysis experiments indicated that PSiMc might be involved actively in the redox turnover of the RC photochemistry. Well-measurable photocurrent can be recorded even after couple of weeks incubation time of the sample. An increase in the photocurrent, after successive illumination, might be due to the accumulation of charge carriers (note, that the quinone mediator, ubiquinone-0 is present), however, this idea still needs to be verified. We also prove that RC can be docked to cytochrome c, which is attached chemically to the PSiMc. This finding indicates that the docking site of the cytochrome remains accessible to the RC after the binding procedure.

References
M. R. Jones, Biochem. Soc. Trans., 37, 400-407. DOI: 10.1042/BST0370400, (2009);

Acknowledgements
This work was supported by grants from the bilateral agreement of Hungarian Academy of Science and CONACYT (Mexico, No. 801), from PHOTOTECH TD1102 COST project and from the TÁMOP-4.2.2.A-11/1/KONV-2012-0060.
ORAL PRESENTATIONS

LASER INDUCED FORWARD TRANSFER (LIFT) FUNCTIONALIZATION OF SCREEN-PRINTED ELECTRODES WITH BACTERIAL REACTION CENTRES FOR MEDIATORLESS HERBICIDE BIOSENSING

L. GIOTTA¹, M. CHATZIPETROU², D. CHIRIZZI¹, M. TROTTA³, M. MASSAOUTI², M.R. GUASCITO¹, F. MILANO³, I. ZERGIOTI²

¹DiSTeBA, Università del Salento, S.P. Lecce-Monteroni, 73100 Lecce, Italy.
²Department of Physics, NTUA, Iroon Polytechniou 9, Zografou, 15780 Athens, Greece
³CNR-IPCF, UOS Bari, Via Orabona 4, 70126 Bari, Italy.

The fabrication of low-cost, stable, sensitive and selective biosensors for herbicides has gained considerable attention in recent years as modern agriculture makes massive use of pesticides that are known to be harmful for human health. The use of photosynthetic proteins for herbicide detection is the most straightforward strategy for this aim, as these are the natural target of this class of chemicals. Among the possible biosensor devices, the most successful that have led to commercial products are based on amperometric detection. In particular, in this type of biosensors, the monitored photocurrent is progressively attenuated in the presence of increasing amount of analyte, minimizing the mathematical treatment of the data. In the present work, we show the functionalization of screen-printed electrodes with an ordered film of photoactive biological material, by means of laser induced forward transfer (LIFT) technique, in order to fabricate a biohybrid device for energy conversion and/or biosensing. LIFT is an advanced tool for achieving the direct immobilization of biosystems [1] with high spatial resolution, due to the high impact pressure of the transferred droplets, at the receiver substrate. As a result, it enhances physical adsorption onto the electrode surface and high photocurrents can be attained by using extremely low quantities of deposited samples. The biomaterial used is the photosynthetic reaction center (RC) from the bacterium Rhodobacter (Rb.) sphaeroides. Cathodic or anodic photocurrents are detected depending on the potential applied to the working electrode: in particular, cathodic photocurrents are detected at the donor side under reducing potentials and anodic ones at the acceptor side under oxidizing potentials. Rb. sphaeroides RC is classified as Q-type and resembles the more evolved photosystem II (PSII) found in plants, algae and cyanobacteria, sharing the sensitivity to the same class of herbicides. However, bacterial RC has a simpler architecture, is more stable and is more selective to a particular class of herbicides, the triazinic ones, while PSII is sensitive to different classes of pesticides [2]. In the present case, thanks to the intimate contact between the RC and the electrode, we could detect cathodic photocurrents in the absence of any mediator at both acceptor and donor side, bringing about several advantages: simpler biosensor architecture, ideal square-wave shape of the photocurrents, and independence on the mediator concentration. Most importantly, the absence of quinone, often used as mediator at the acceptor side makes the biosensor particularly sensitive to the herbicide tested, due to the competitive nature of the binding reaction. We tested the herbicide terbutryn which, although is no longer permitted in the EU, has the strongest binding affinity to the RC and is more suitable to show the potentiality of the final device. A limit of detection for the terbutryn was found in the range 8-30 nM which is slightly higher than the maximum level of pesticide permitted by the EU laws for drinking water. Further optimization of the protein deposition or protein engineering is required in order to completely meet the biosensor performance with the requested sensitivity.

References


Humankind is in great need of new energy sources. The use of solar radiation for powering the planet would fulfill the energy requirements of Earth’s inhabitants as well as greatly mitigate tension flares arising from the uneven distribution of fossil fuels and environmental problems associated to their extraction procedures. How to proceed then? Easy to say! Mother Nature is inspiring: all life on earth is based on the conversion of the solar radiation into high energy molecules, including gas and oil human beings are consuming these days, by mean of the so-called primary photoconverters, i.e. the photosynthetic organisms, plants, algae and some kind of bacteria. So, let’s learn from Nature and assemble in our laboratories artificial systems capable of exploiting solar energy for photocatalysis and electrical energy production [1], i.e. mimic photosynthesis. Not an easy task of course, but a large number of laboratory are heavily involved since the last 25 years in the field of artificial photosynthesis and are obtaining encouraging results. The photosynthetic apparatus used by photosynthetic organisms to convert solar energy and drive their metabolism is the photochemical core where photoconversion takes place, and is constituted by a protein portion allocating several pigments directly involved in the harvesting of solar light and in the subsequent sequence of electron transfer reactions which eventually lead to the formation of an electron-hole couple to be used for any energy requiring process. In artificial photosynthesis the role of the protein scaffold in often ignored and attention is devoted to assembly molecular system for optimising light harvest and electron-transfer reactions, focussing to the “less-complex” portion of the photosynthetic apparatus [2]. What would be a different paradigm in artificial photosynthesis? Assemble artificial photoconverters using genuine natural components formed by hybrid organic-biologic systems [3]. The hybrids have a central protein, the so-called photosynthetic reaction center (RC) that converts sunlight into a charge-separated state having a lifetime sufficient to allow ancillary chemistry to take place. The RCs can be eventually garnished with opportune organic moieties to be used for different applications [4,5]. The state of the art of these hybrid organic-biologic photosynthetic assemblies will be reviewed.

References
ORAL PRESENTATIONS

THE USE OF ELECTROCHEMICAL TRANSDUCTION IN DEVELOPING PSII BASED BIOSENSORS - PRO’S AND CON’S
SIMONA CARMEN LITESCU¹, IOANA VASILESCU¹, SANDRA EREMIA³, G.L.RADU¹, IOAN BALINT², FLORICA PAPA², VIVIANA SCOGNAMIGLIO³, AMINA ANTONACCI³, MAYA LAMBREVA³, GIUSEPPINA REA³
¹ National Institute of Research and Development for Biological Sciences Bucharest, Centre of bioanalysis
² Institute of Physical Chemistry of the Romanian Academy “I.G Murgulescu”
³ Institute of Crystallography, Italian National Council of Research

There is an increasing demand worldwide for low cost, fast, and reliable methods for monitoring chemical species, additives, and xenobiotics in clinical chemistry, environmental sciences, and food related processes. Chemical sensors and biosensors offer all these advantages since they can be easily used in both laboratory and field applications.

In developing sensors and biosensors one of the key-issues is related to the capability of designing the perfect match between appropriate transduction (to ensure the signal sensitivity) and biorecognition element functionality (to ensure specific discrimination of the targeted analyte, sensitivity and response stability). The use of signal amplifiers as nanoparticles/nanowires in order to enhance the electrochemical response will be discussed and exemplified for Pt@M bimetallic nanoparticles and Carbon Nanofibres-Gold Nanoparticles. The exploitation of PSII enriched membranes is particularly suitable for the development of electrochemical biosensors as the enzyme is selectively activated by light consequently minimizing the electrochemical interferences¹,². The use of different photosynthetic strains will be discussed and the obtained results will be compared for various electroactive surfaces supports. In order to ensure the biosensor functionality it should be maintained the cell viability which will ensure the proper photosynthetic activity. Consequently, when designing a biosensor one of the critical points is the bio-recognition element immobilization, since the immobilization must preserve, or, if possible improve, the bio-mediator activity. Results obtained by using two immobilization protocols will be presented, influence of material load, temperature, pH variation, etc. being discussed.

The results obtained using a new developed PSII-Pt@Au_CSPE amperometric biosensors applied in assessing the urban water contaminants will be presented; the optimum response was characterized by a sensitivity of 15nA/µmolL⁻¹ for TCE/PCE contaminants.

References

Acknowledgements
Work was partially supported by project New BImetallic nanoparticles with applications in water CLEANing of chlorinated compounds and BiOSensors, BICLEANBIOS, PCCA 1, contract 46/2012.COST ACTION TD1102 is acknowledged for providing the opportunity of developing common researches and creating a stimulating working environment.
GREEN ORGANISMS TO THE RESCUE: THE POTENTIAL OF CYANOBACTERIA, ALGAE, AND HIGHER PLANTS FOR THE REMEDIATION OF RADIOACTIVELY CONTAMINATED WATERS

PAUL JANSSEN¹, NATHALIE VANHOUDT², TALAL AL MAHAINI², NATALIE LEYS¹, HILDEGARDE VANDENHOVE²

¹Microbiology, Belgian Nuclear Research Centre SCK•CEN, Mol, 2400, Belgium
²Biosphere Impact Studies, Belgian Nuclear Research Centre SCK•CEN, Mol, 2400, Belgium

The potential of photosynthetic “green” organisms to effectively remediate water masses contaminated with radionuclides was evaluated for scenarios related to nuclear installations and included the following radionuclides: ¹³⁷Cs, ¹³⁴Cs, ¹³⁶Cs, ⁹⁰Sr, ¹³¹I, Pu, Am, ¹³²Te/¹³²I, ⁵⁶Co, ⁶⁰Co, ⁵¹Cr, ¹¹⁰Ag, ⁵⁴Mn, ¹²⁴Sb, ⁵⁹Fe, ⁶⁵Zn, ⁹⁵Zr, and ⁹⁵Nb. An extensive literature review was undertaken in respect to the above metals for terrestrial and aquatic plants (TP/AP), macro- and microalgae (MA/mA), cyanobacteria (CB), and dead biomass, with attention to culturing conditions, chemical pre-treatments of the biomass, metal-sorbent contact time, process conditions, maximal removal and bioconcentration for one or more metals (i.e., in mixed form), system layout, robustness, and performance, and desorption capacity. A database with 440 entries was built for the 15 elements listed above and a total of 266 living organisms (TP, 63; AP, 139, MA, 9; mA, 15; CB, 40) and 120 types of dead biomass. From these data, 29 organisms (either alive or as dead biomass) were selected. This selection was primarily based on radionuclide uptake, bioconcentration factor, and removal rate. For each of these selected organisms, we identified minimal and optimal environmental conditions and evaluated to what extent certain environmental parameters (such as pH, temperature, light, salinity, borate content, presence of biocides, etc.) may influence the actual processes of bioaccumulation and biosorption.

Because of the intrinsic limitations of a literature review, no matter how extensive, selected organisms and sorbents need to be tested for their performance under realistic conditions (most of the literature data were obtained under specific laboratory conditions that deviate from in situ conditions). In order to draft a shortlist and perform an objective ranking among the selected organisms, we applied a scoring guide for pairwise comparison taking into account two selection categories, i.e. system performance and system robustness, each category holding a number of criteria with distinct weights and scores. The obtained results were then visualised for each ranked organism in individual SWOT analysis graphs. Finally, these graphs are now used by us to formulate organism- and system-specific solutions and to devise corresponding next-step experiments.
ESR Oral Presentations
**RHODOBACTER SPHAEROIDES CONVERTS LIGHT INTO ELECTRICITY IN A NEW DESIGNED MICROBIAL FUEL CELL BY ACETATE PHOTO-FERMENTATION**

BILGE HILAL CADIRICI
Bioengineering Department, Gaziosmanpasa University, Tokat, Turkey

Photosynthetic bacteria are known as the most powerful hydrogen producers. Photosynthetic bacteria can produce H₂ by consuming organic acids. Unlike dark fermentation, photo-fermentation only proceeds in the presence of light. Fuel cells are electrochemical energy conversion devices which converts hydrogen and oxygen into water to generate electricity. In this study we combine the biohydrogen from *R. sphaeroides* with the subsequent conversion to electricity. With this purpose, we design a 50 mL volume PMFC with platine cathode and carbon anode. We used potassium permanganate as electron acceptor in cathodic cell. Acetate was used as carbon source in growth medium for *R. sphaeroides* anoxygenic photosynthesis. In order to check the efficiency of the fuel cell, we compare the electrical potential producing capacity of *R. sphaeroides* with the published papers. It was shown that highest electricity potential of *R. sphaeroides* we gain is 1 Volt with 78.96 µA current at 48 h. The power density is 37.83 mW/m² have max 6 mW/m² power density with Cyanobacteria (Lyngbia).

References
Great interest has been given to photosynthetic systems for their potential use in future technological applications such as bio-sensors and bio-solar cells. Photosystem I offers a fast electron transfer rate and a high light induced redox potential difference making it desirable for photovoltaic devices. Photosystem I can be found in plants and cyanobacteria and although they share a lot similar properties they differ mainly in their antenna size. This difference in antenna size may play a big role in their photocurrent generation when incorporated in a bio-photovoltaic device. In this work I will compare the photocurrents generated by photosystem I from spinach and cyanobacteria on a conductive surface.
DIFFERENT APPROACHES FOR CONTACTING PHOTOSYSTEM I WITH ELECTRODES FOR EFFICIENT PHOTOCURRENT GENERATION


1Biosystems Technology, Institute of Applied Life Sciences, Technical University of Applied Sciences, 15745 Wildau, Germany
2Biochemistry and Structural Biology, Institute for Biology, Humboldt-University of Berlin Unter den Linden 6, 10099 Berlin, Germany
3Institute for Molecular, Cell & Systems Biology, Glasgow Biomedical Research Centre, University of Glasgow, 120 University Place, Glasgow, Scotland, UK

The efficient coupling of photosystem I (PSI) with electrodes for biologically inspired light-to-current converting devices is becoming the focus of current research[1]. Here, highly interdisciplinary approaches are demanded. However, these solar energy devices are only suitable for future sustainable and renewable applications, if many issues, such as photocurrent efficiency, biocompatibility and stability, can be solved. To address these problems, we have developed and advanced two basic approaches relying on an adsorption strategy for the rather unidirectional deposition of PSI on electrodes. (I) By the use of cyt c acting as a wiring agent and simultaneously as assembly template, we have demonstrated a mono- and multilayer system, working as an efficient biomolecular photocathode[2]. Furthermore we could invent a self-growth assembly mechanism as well as the construction of 3D architectures by incorporation of DNA for higher photocurrent generation and improved stability. (II) Another photocathode approach has also been shown by us including the directed assembly of PSI on the nanomaterial graphene[3]. Here, different π-systems were investigated in order to allow an efficient deposition so as an electrochemical connection of PSI to graphene. The nature of the chosen π-systems has a pronounced effect on the electrode behaviour. By this strategy we have developed highly light-harvesting electrodes even when no additional potential was applied. The photocurrent density can be additionally enhanced when the electron withdrawal is enforced at the stromal side of PSI. The two approaches result both in a rather unidirectional photocurrent generation. The protein-based electrode provides a good basis for the construction of further advanced bio-inspired systems. Whereas the graphene-based strategy has clear benefits in the light-to-current conversion efficiency and is suitable for high performance bio-photovoltaic devices.

References

Acknowledgements
Bundesministerium für Bildung und Forschung (BMBF), Germany (Biotechnologie 2020+, projects: 031A154A+B) and BBSRC/EuroCores (BB/J00823011) are kindly acknowledged.
We have recently tailored conducting metal oxide electrodes to significantly improve the integration of redox active enzymes into photoelectrochemical (PEC) cells.\(^1\) The hierarchically structured inverse-opal mesoporous indium-tin oxide (ITO) electrode used this study offers a much larger accessible electroactive surface for enzymes anchoring than conventional flat or mesoporous electrodes.\(^2,3\) Furthermore, the dimensions of these electrodes can be easily tuned to host a wide range of guests. In this study, Photosystem II (PSII) from *Thermosynechococcus elongatus* and hydrogenase from *Desulfomicrobium baculatum* was adsorbed on the hierarchically structured electrodes to give rise to unprecedentedly high and stable enzyme loading. Subsequently, large (photo)current densities were measured, which enabled accurate \(\text{O}_2\) and \(\text{H}_2\) product detection. When the two enzymes were wired together with the aid of an applied bias, the semi-artificial cell demonstrated quantitative electron flow from PSII to the hydrogenase with the production of \(\text{H}_2\) and \(\text{O}_2\) being in the expected two-to-one ratio. A light-to-hydrogen conversion efficiency of 5.4% under low intensity red-light irradiation was achieved. This is the first reported PEC cell that wires PSII to hydrogenase for complete light-driven water splitting,\(^1\) and comparisons of this system against other water-splitting bio-PEC systems will be drawn during this presentation.

References

Acknowledgements
This work was supported by the U.K. Engineering and Physical Sciences Research Council, the U.K. Biology and Biotechnological Sciences Research Council, a Marie Curie Curie International Incoming Fellowship.
BINDING OF *RHODOBACTER SPHAEROIDES* REACTION CENTRES TO TiO$_2$ NANOPARTICLES

M. KUJAWA$^1$, R. BIAŁEK$^2$, M. JONES$^3$, K. GIBASIEWICZ$^2$

$^1$ Adam Mickiewicz University, Faculty of Physics, Poznań, Poland,
$^2$ Adam Mickiewicz University, Faculty of Physics, Poznań, Poland
$^3$ School of Biochemistry, Medical Sciences Building, University of Bristol, Bristol, UK

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 RCs 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$_2$ nanoparticles. Additional objective was to determine the possibility of obtaining a stabilized TiO$_2$ solution, native to RC. For this purpose Fluorescence Correlation Spectroscopy (FCS) of RCs with different TiO$_2$ nanoparticles in solution was applied. By using this method one can obtain information about the size of the complex of RCs and TiO$_2$ nanoparticles.

References
ESR ORAL PRESENTATION

POLARIZED LIGHT SPECTROSCOPY OF REACTION CENTERS FROM RHODOBACTER SPHAEROIDES

MÁRTA DOROGI¹, MELINDA MAGYAR², GÁBOR SIPKA², GYÖZÖ GARAB¹, LÁSZLÓ NAGY², PETAR H. LAMBREV¹

¹ Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary
² Department of Medical Physics and Informatics, University of Szeged, Szeged, Hungary

Photosynthetic reaction center (RC) complexes are membrane pigment-protein complexes carrying out the primary photochemical solar energy conversion in photosynthesis. The general RC architecture is similar for all photosynthetic organisms – from anoxygenic bacteria to green plants. The RCs of anoxygenic purple nonsulfur bacteria, such as Rhodobacter sphaeroides, share their basic structure with Photosystem II in plants but are simpler in their biochemical composition and spectroscopic properties. The primary photophysical and photochemical reactions of photosynthesis are best understood in these RCs [1]. Current biotechnology, bioengineering and rational design methods make bacterial RCs prospective for developing bionanodevices. In this respect, a thorough understanding of the structure-function relationships, photophysics and spectroscopy of the RC is paramount. With this work we aimed to complement the existing spectroscopic characterisation of bacterial RCs in order to create sound experimental base for understanding their photophysical properties from first principles. To this end we measured linear spectra of isolated RCs of R. sphaeroides strain R-26 in the visible and near-infrared region – absorption and circular dichroism (CD) spectra of isotropic solutions of RCs, as well as linear dichroism and anisotropic CD (ACD) spectra of RCs with a fixed spatial orientation. Absorption and LD spectra reveal excited state levels of the RC pigments (4 bacteriochlorophylls – PM/L, BM/L and 2 bacteriopheophytins – HM/L), and relative magnitudes and directions of the corresponding ground to excited state transition dipole moments. However, unambiguous assignment of molecular transitions is extremely difficult based on absorption spectra alone [2]. The excited-state reactions of the RC critically depend on interactions between the pigments creating delocalized exciton states. CD spectra are sensitive to exciton interactions but are difficult to analyse as they typically consist of largely overlapping positive and negative bands. ACD spectroscopy, applied here for the first time on RCs, provides additional information by enabling the selective probing of transitions based on their geometry (orientation of the transition dipole moment). The well-known RC absorption spectrum has three bands in the near-infrared region, at ~750, 800 and 870 nm [3], denoted H, M, and P, respectively. The P band corresponds to excitation of the special pair bacteriochlorophylls PM/L, the H band – to excitation of the bacteriopheophytins, and the M band encompasses transitions involving all bacteriochlorophylls (PM/L and BM/L). The CD spectrum has well discerned bands at 750 and 870 nm and a split positive-negative shape in the M-band region, characteristic for exciton interactions between pigments. The ACD spectrum measured with light perpendicular to the plane of membrane suppressed the magnitude of the (-) 815 nm CD band, indicating that the corresponding transition dipole moment is (predominantly) perpendicular to the membrane plane, while at the same time revealing the existence of another CD band appearing as a shoulder at 825 nm. Thus, the data reflect exciton interactions between P and B pigments in the RC and provide additional geometric constrains that can help in the assignment of excited states and electronic transitions to bands in the experimental optical spectra.

References

Acknowledgements
This work was supported by grants from Switzerland through the Swiss Contribution (SH/7/2/20), TÁMOP-422D-15/1/KONV-2015-0024, and European Cooperation in Science and Technology network, PHOTOTECH COST TD1102.
The green alga, *Chlamydomonas reinhardtii*, is capable of sustained hydrogen photoproduction during about one week when incubated as batch suspension culture in the absence of sulfur [1]. Cell immobilization within a thin Ca-alginate film was shown to improve hydrogen yield due to increase in the rate and duration of photoproduction under S depletion [2,3]. In our current experiments we optimized the immobilization procedure so as to make hydrogen photoproduction to last up to three weeks; in this case hydrogen production is limited mainly by exhaustion of intracellular starch reserves and disruption of alginate hydrogel structure. We first studied the effects of different wavelengths on hydrogen photoproduction and showed that illumination by blue light at 435 nm or red light with maximum at 690 nm favoring PSI excitation improved the stability of photosystem II centers and enhanced the rate of hydrogen evolution during the first week of S deficiency. The optimal light intensity for hydrogen photoproduction has been confirmed to be about 40 μmol photons m⁻² s⁻¹. Stronger light accelerated hydrogen evolution but shortened the duration of the process, whereas exposure to weaker light diminished the rate of hydrogen evolution. Initial medium pH values in a range 6.5–7.0 were found to be optimal for hydrogen photoproduction mainly due to enhanced accumulation of starch reserves.

References


Catalysts based on earth abundant elements for \( \text{H}_2 \) oxidation or evolution are central to the development of alternative technology for energy conversion schemes. However, such catalysts are often fragile and undergo various deactivation pathways under conditions relevant to technological applications. We proposed a concept based on a viologen modified hydrogel to protect such catalysts [1]. The hydrogel serves as redox buffer that quenches deactivating pathway and as redox matrix to electronically contact the catalyst to the (photo-)electrode. For the latter function, the formal potential of the viologen has to be adjusted to the catalyst and the direction of the electron transfer. Viologens were investigated as redox mediators since their potential can be adjusted via electron donating or electron withdrawing groups. Changing the substituents at the viologen affects both the solubility of the cation radical and the reversible potential. We study the effect of various substituents on the properties of both 2,2'- and 4,4'-viologens. Moreover, the viologens were coupled to polymer backbones via the reaction of thioisocyanate or isocyanate groups with alcohol or amine functionalized polymers. Crosslinking of the viologen modified polymers allows the immobilization of redox catalysts such as hydrogenase on (photo-)electrodes. The hydrogen oxidizing anode is based on [NiFe]- or [FeFe]- hydrogenase and a viologen modified hydrogel with a potential of -420 mV vs Ag/AgCl, which is positive to the standard redox potential of the \( \text{H}^+/\text{H}_2 \) (-0.641 V vs Ag/AgCl at pH 7) [2,3]. In order to achieve hydrogen generation we develop a polymer with a redox potential negative to -0.641 V.

References
The use of solar light is mandatory in order to scale-up photobiological hydrogen production to an industrial level. Several photobioreactor designs are being proposed in mass culture of microalgae, while information on the scale-up of hydrogen production is still limited. The most important problem of the culture performance outdoors is light saturation. To this end we assessed the performance of two PBRs (50L and 1300L) devised and constructed by our group. The reactors have been tested for the outdoor cultivation and hydrogen production using the cyanobacterium *Synechocystis* PCC 6803. To scale-up the process with large outdoor cultivation systems a better understanding on how the organism reacts to environmental factors, in particular to light and temperature is crucial. Experiments were carried out on growing cultures at different cell concentrations in order to test the robustness of *Synechocystis* to the changes in light intensity that occur during the day.
The main focus of our work is to understand the structural underpinnings, dynamics and pathways of excitation energy transfer (EET) in and between pigment-protein complexes in photosynthetic membranes. EET is the initial step of photosynthesis, universal among all photosynthetic systems, that funnels absorbed energy towards the photochemical reaction centres. Fast and efficient EET is a prerequisite for efficiency in solar energy conversion. In plants, the two photosystems – Photosystem I (PSI) and Photosystem II (PSII) have their own separate light-harvesting systems whose function is coordinated and finely regulated to achieve balanced energy flow to the photosystems and prevent overexcitation under conditions of excess light. Regulation of energy flow in the thylakoid membrane involves structural changes at the level of single protein complexes, interactions of supercomplexes and overall remodeling of the membrane architecture.

To study such complex processes occurring in nature we employ model membranes that enable us to reduce the complexity of the system, identify the minimal units and interactions involved in a particular process. In this work we isolated light-harvesting complex II (LHCII) and reconstituted it in liposomes of native plant lipids. We probed the excitation dynamics in the complexes and EET between complexes by ultrafast two-dimensional optical spectroscopy and time-resolved fluorescence spectroscopy, and compared with solubilized and aggregated LHCII. The results showed no significance perturbations in the EET within the LHCII complexes when they are reconstituted in proteoliposomes however the overall excitation lifetime was shorter than in detergent solution. In the membrane there was efficient EET between LHCII supercomplexes with domain sizes comparable to native thylakoid membranes.

The ability of LHCII to transfer absorbed energy to PSI and thus increase the effective antenna size of PSI was tested in reconstituted PSI:LHCII membranes. In proteoliposomes reconstituted at varying ratios of PSI to LHCII, EET between LHCII and PSI was confirmed by steady-state and time-resolved fluorescence spectra, however the results also showed the existence of LHCII domains not energetically connected to PSI. Due to the very fast charge separation dynamics in PSI, binding of additional LHCII units appeared to have very little impact on the photochemical quantum yield of PSI.

Acknowledgements
This work was supported by grants from the Hungarian National Research Fund This work was supported by the Hungarian Scientific Research Fund (OTKA-PD 104530, TÁMOP-4.2.2.A-11/1/KONV-2012-0060), Hungarian National Innovation Office and A*STAR Singapore (NIH-A*STAR TET_10-1-2011-0279).
PHOTOINHIBITION EXPERIMENTS IN NATURAL ACCESSIONS OF ARABIDOPSIS THALIANA SUGGEST POTENTIAL FOR IMPROVEMENT IN THE LIFE TIME OF PSII BASED BIO-DEVICES

HETA MATTILA, KUMUD B. MISHRA, ANAMIKA MISHRA, KATEŘINA NOVOTNA, KAREL KLEM, ESA TYYŞTJÄRVI

1Department of Biochemistry / Molecular Plant Biology, University of Turku, 20014 Turku, Finland
2CzechGlobe - Global Change Research Centre AS CR, v.v.i., Bělidla 986/4a 603 00 Brno, Czech Republic

Photoinhibition and production of singlet oxygen are closely related to each other and limit the lifetime of PSII based bio-devices/sensors. It is well known that the cold tolerance of PSII differs in plants originating from warm and cold climates; however, the natural variation in photo-inhibition of cold-sensitive and tolerant plants has not been systematically investigated. In order to understand tolerance to photoinhibition in different PSII systems, we did experiments on non-acclimated (8 weeks old plants grown in day/night temperature 22 °C/18 °C) and cold acclimated plants (obtained by transferring non-acclimated plants to 4 °C after 6 weeks of growth). The seeds came from four natural accessions of A. thaliana: Tenela (Te, Finland), Rschew (Rsch, Russia), Columbia-0 (Col-0, genetically related to an accession from Germany) and Coimbra (Co, Portugal). Among the studied accessions; Rsch showed high tolerance to photoinhibition and leaves of Co were sensitive in both acclimated and non-acclimated state. The photoinhibition tolerance of accessions Col-0 and Te was between the sensitive and tolerant accessions in both acclimated and non-acclimated states. Cold acclimation increased flavonoid content, modified the xanthophyll cycle and shifted the light harvesting complexes from PSII toward PSI and further improved the tolerance to photoinhibition in all used accessions of A. thaliana. We demonstrate that proper selection of species and cold acclimation can significantly improve the lifetime of PSII based bio-devices.

Acknowledgements
HM thanks COST action project TD1102 for providing STSM grant to perform experiments at CzechGlobe center Brno. ET and HM were also supported by Academy of Finland. KBM, AM, KN and KK acknowledge projects: ENVIMET (CZ.1.07/2.3.00/20.0246) and L01415, for support.
LASER INDUCED FORWARD TRANSFER: A NOVEL TECHNIQUE FOR PRINTING BIOMATERIALS
MARIAPA MALLA MALLA, MARIANNEZA CHATZIPETROU1, GEORGE TSEKENIS1, FRANCESCO MILANO3, IOANNA ZERGIOTI1
1National Technical University of Athens, Physics Department, Iroon Polytechneiou 9, 15780 Zografou, Athens, Greece
2Biomedical Research Foundation of the Academy of Athens, Soranou Ephessiou 4, 11527 Athens, Greece
3National Research Council, Istituto per i Processi Chimico-Fisici, c/o Chemistry Department, Via Orabona 4, 70126 Bari, Italy

During the last decade, laser based techniques have received significant attention as direct, cost-effective and high resolution printing/patterning techniques applied in a variety of applications including sensors, cell printing and microeletronic devices. Among them, Laser Induced Forward Transfer (LIFT) exhibits inherent advantages, in terms of high spatial resolution of the printed and/or processed patterns, enabling the maskless (direct) deposition of highly resolved features of biomaterials including of proteins, enzymes, DNA, cells, virus etc., which is essential in various fields and applications including pharmaceutical, bioengineering, and sensing applications. The versatility that LIFT presents, offers the capability of its implementation for the fabrication of a variety of sensing devices, i.e. resistive, amperometric, transistor-based optical sensing, and on different substrates. One of the key advantages of LIFT is that can be used as an advanced process for functionalizing the sensors surface without the need for intermediate layers, or chemical functionalization techniques, that often require treatment with toxic solvents and hazardous chemicals. Update, the LIFT technique has been applied for the fabrication of several types of biosensors with high spatial resolution in a direct approach. In this work, examples of chemical and biosensors fabricated based on the LIFT technique will be presented. In particular, we will present recent results on LIFT immobilization of the photosynthetic reaction center (RC) from the bacterium Rhodobacter (Rb) onto Screen Printed Electrodes (SPEs) for the detection of the herbicide terbutryn [4]. In the framework of developing, a sensitive biophotonic sensing system, a novel approach for the immobilization of aptameric sequences -targeting analytes such as mycotoxins, heavy metals, and other- on modified silicon nitride surfaces- based on the laser induced transfer technique will be shown.

References

Acknowledgements
This work is supported by the European Research Council under the European Community’s Seventh Framework Program (FP7/2007–2013) COST Action TD1102 Photosynthetic proteins for technological applications: biosensors and biochips (PHOTOTECH) and This work is supported by the EC funding FP7 ICT-2013 BIOFOS project.
Posters
Photovoltaic devices were fabricated using micro lithography. The devices were made of sub-micron transparent ITO bottom electrode and cross top metal electrode in a maze configuration. Oriented multilayers of PSI were fabricated on the ITO bottom electrode. The oriented PSI multilayers were formed by the use of bifunctional cross linker molecules attached on one end to free amino groups at the top of PSI layer and to unique cysteine mutants at the bottom of successive PSI layers.

References
One of the biggest problems of the contemporary world is the depletion of fossil fuels. Among possible solutions for meeting future energy demands, solar cells based on photosynthetic reaction centers (RCs) of the purple bacterium *Rhodobacter sphaeroides* are considered. The proposed construction is similar to that of Dye Sensitized Solar Cells invented by Michael Graetzel, which consist of a TiO$_2$ mesoporous layer covered with a photoactive dye, but where natural pigment-proteins are used instead of the artificial dye [1]. For the described research, new genetically engineered RCs were used in which a titanium dioxide binding peptide tag was added. For construction of solar cells, two types of TiO$_2$ paste were used: a ready-made one containing 20 nm particles and a homemade paste with 50 nm particles. Also two types of electrolytes were compared: sodium dithionite and TMPD. Steady-state absorption spectra of RCs on TiO$_2$ substrates are presented to prove the effectiveness of binding of RCs. Photocurrents generated were either positive (injection of electrons from RCs to substrate) or negative (injection of electrons from substrate to RCs) depending on the composition of the electrolyte solution. Values of the currents obtained were of the order of 100 nA to a few microamperes. For explanation of the observed photocurrents, a mechanism connecting fractions of open and closed RCs was proposed. Negative photocurrent was attributed to RCs in the “open state” whereas the positive one – to RCs in “closed state”. Understanding of this mechanism will hopefully enable further optimization of our prototype solar cell. It is proposed by Lukashev et al. [2] that the electron is injected from the triplet state in RC so spectroscopic results of mutants with different triplet formation yield will be presented.

References

Acknowledgements
We acknowledge financial support from the Polish government and European Union (project entitled “Construction of photovoltaic cells based on *Rhodobacter sphaeroides* reaction centers” no. 18/POIG/GP/2013).
P03 - PHOSPHORYLATED LHB2 HOLDS A SPECIFIC ROLE IN PSI-LHCII SUPERCOMPLEX ASSOCIATION DURING STATE TRANSITIONS IN ARABIDOPSIS

AURELIE CREPIN, STEFANO CAFFARRI
Aix Marseille Université, CEA, CNRS, UMR 7265 Biologie Végétale et Microbiologie Environnementales, Lab. de Génétique et Biophysique des Plantes, 13009 Marseille, France

State transitions are an important short-term response to changes in light quality or quantity that maintains the excitation balance between Photosystems I (PSI) and II (PSII). They consist in a reversible transfer of LHCII antennas (the main antennas of PSII) from one photosystem to the other, depending on light conditions. In plants, under a light favoring PSII, the LHCII heterotrimer is phosphorylated and moves to PSI to adjust the excitation balance of the two photosystems (State II). On the contrary, if PSI is preferentially excited, the trimer is dephosphorylated and moves back to PSII (State I). LHCII trimers that stay attached to PSII can also be phosphorylated [1]. The phosphorylation of the antennas has been well studied in a qualitative way, as well the kinetics of the mechanism [2], but the absolute phosphorylation level of the LHCII and its isoforms remains to be determined. In this work, we quantified the phosphorylation of the Lhcb1 and Lhcb2 isoforms composing LHCII in PSI-LHCII and PSII-LHCII supercomplexes in WT and state transitions mutants of Arabidopsis thaliana. We found that around 40% of the Lhcb1-2 monomers are phosphorylated in PSI-LHCII. The percentage is only 15-20% in PSII-LHCII. Dephosphorylation assays with a recombinant PPH1/TAP38 phosphatase [3, 4] permitted us to further investigate the role of each isoform in the state transitions phenomenon. The results suggest that a single phosphorylated Lhcb2 is sufficient for the stable association of the LHCII trimer to PSI to form the PSI-LHCII supercomplex. These findings highlight the different roles of the Lhcb isoforms in the regulation of photosynthesis and the optimization of the electron transport chain, and are a step towards a better understanding of the short-term response to changes in light conditions in plants.

References

Acknowledgements
The work has been supported by the French National Research Agency Grant ANR-12-JSV8-0001-01.
Phenazine methosulfate (PMS, 5-Methylphenazinium methyl sulphate) is a redox mediator widely used in studies of energy and electron transfer in photosystem I (PSI). It has been also applied in biohybrid constructions using photosynthetic systems as catalysts for solar energy conversion and biofuel production.

PSI is a photosynthetic protein-pigment complex, commonly found in plants, algae and cyanobacteria. In these organisms it cooperates with photosystem II (PSII) in the light-driven transfer of electrons from the water molecule to the NADP⁺ molecule, which is next used to reduce the carbon dioxide into carbohydrates in the light-independent phase of photosynthesis. Pigments form the antenna systems that capture sunlight energy and transfer it to the reaction centers (RC), where it is used to initiate charge separation. The beginning of PSI electron transport chain (ETC) is a heterodimer of chlorophyll a and chlorophyll a', traditionally called P700, due to the peak wavelength of its absorption. At the initial stage of charge separation, P700 is oxidized to P700⁺. RC is closed in this state and unable to conduct next electron transfer. In natural conditions (in vivo), P700⁺ is reduced to P700 by plastocyanin, which transports electrons derived from PSII. As a result, the RC is re-opened and another light-stimulated charge separation becomes possible. In laboratory conditions, to artificially open the reaction centers in isolated PSI complexes (in vitro), reducing agents are added to the solution. PMS reduced by sodium ascorbate is the most popular one. The spectral and redox properties of PMS are, however, fairly complex [1,2]. The binding site for electrons in redox reactions is 5-methylphenazine (MP) that may occur in the oxidized form as 5-methylphenazinium ion (MP⁺), in the two-electron reduced form (MPH) or in the intermediate semiquinonoid forms (radicals MP⁻ and MPH⁺⁻). Therefore, we performed a series of the steady-state absorption measurement to identify the redox forms of MP in the various mixtures of PMS with ascorbate and/or extract of photosynthetic pigments. Redox mediators, that keeps reaction centers open or are used in photovoltaic cells and in biofuel production, should not derive excitation energy from the investigated or employed photosynthetic systems. It has recently been contested whether PMS meets that condition [3]. It was shown by steady-state fluorescence measurements that addition of PMS decreases intensity of light emitted by chlorophylls embedded in PSI complexes. It was claimed that this effect is caused by quenching of chlorophylls' excited states by PMS. However, it has not been explained so far what is a mechanism and the rate of observed quenching phenomena. Thus, we decided to test the effect of PMS on excited states of chlorophylls in solution and in PSI using not only the steady-state but also time-resolved fluorescence spectroscopy method (time-correlated single photon counting). We applied Stern-Volmer model to obtain quenching rate of free pigments as well as pigments associated with PSI.

References
Hexavalent chromium represents an outstanding risk for the environment and the health of human beings, as it is considerably involved in the genesis of cancer and other fatal diseases. Biological reduction of Cr(VI) to Cr(III) is an interesting process that could be exploited for environmental applications such as biosensing and bioremediation. The photosynthetic purple bacterium *Rhodobacter sphaeroides* is known for its ability to tolerate high concentrations of several heavy metal ions, to bioaccumulate nickel and cobalt, and to reduce oxyanions as tellurite, selenite and chromate. The response of the carotenoidless mutant R26 to chromate stress under phototrophic conditions has been recently investigated by biochemical and spectroscopic measurements, proteomic analysis and cell imaging, revealing interesting Cr(VI) reduction ability associated with morphological and compositional changes of the cell envelope, while no specific stress-induced chromate-reductase activity was found in the soluble proteome [1]. In the present investigation phototrophic biomass of *Rhodobacter sphaeroides* strain R26 was harvested from culture broth, washed, and used as Cr(VI) reduction catalyst. Chromate solutions, buffered at neutral pH and supplemented with succinate and glucose as electron donors, were employed as reaction mixtures. The decrease of Cr(VI) concentration triggered by cells addition was evaluated by the diphenylcarbazide (DPC) assay. The analysis of reaction kinetics showed that *Rhodobacter sphaeroides* resting biomass acts as an excellent bio-catalyst promoting fast chromate reduction in neutral conditions. The role of abiotic variables such as pH, light, temperature and oxygen concentration was also assessed. X-Ray Photoelectron Spectroscopy (XPS) analysis of the bacterial biomass at the end of the reduction reaction revealed the presence of Cr(III) onto the cell envelope, indicating that both Cr(VI) reduction and Cr(III) immobilization processes occur. Cr(III) sequestration by anionic surface functional groups was also confirmed by Fourier Transform Infrared (FTIR) spectroscopy data. These results extend the information available about this phototrophic microorganism and contribute to elucidate its potential in Cr(VI) biosensing and bioremediation applications.

References
Light dependent electron transfer is necessary for photosynthesis but light also damages the photosynthetic machinery. The loss of Photosystem II activity caused by light induced damage is called photoinhibition. By studying the natural variation in photoinhibition we hope to find out how to keep isolated photosynthetic organelles and proteins functional for extended periods of time. We have mapped the reaction kinetics of photoinhibition in several photosynthetic organisms in order to elucidate the damaging mechanisms and photoreceptors of photoinhibition. We have used extensive action spectroscopy on marine cyanobacteria and diatoms, organisms known to differ significantly in their pigment compositions, to study the roles of photosynthetic light harvesting pigments in photoinhibition. Action spectra of photoinhibition were measured in the presence of lincomycin that inhibits PSII repair. The rate constant of photoinhibition was quantified for all wavelengths separately by fitting the measured decay of PSII activity (O₂ evolution and fluorescence) into first order reaction kinetics. Action spectra of PSII were measured from the same organisms. Consistent with earlier photoinhibition studies, the photoinhibitory efficiency of UV radiation was several orders larger than that of visible light in all species [1]. Comparisons of the action spectra of photoinhibition and PSII revealed no clear correlation between these two parameters, which supports the hypothesis that the Mn₄CaO₅ center of the oxygen evolving complex of PSII is the primary photoreceptor of photoinhibition in both UV and visible light [1,2].

Having a solid understanding of the differences in photoinhibition and photosynthetic electron transfer in different species is paramount for the next step in our project in which we aim to characterize photoinhibition and photosynthetic electron transfer routes in the algal chloroplasts of the kleptoplast Sacoglossan sea slugs. These slugs maintain photosynthesis for months within specialized cells lining their digestive track by incorporating chloroplasts from the algae they feed on. This is a remarkable deed, as the animal tissue lacks the genetic machinery that interacts with the chloroplast in the algal cell [3]. The problems of photoinhibition of PSII and the degradation and de novo synthesis of the D1 protein, normally occurring in a time frame shorter than one day, have obviously been solved in the sea slug / chloroplast interaction. We have taken the first steps into characterizing photoinhibition and photosynthetic electron transfer reactions in the sea slug Elysia timida and its algal counterpart Acetabularia acetabulum and next we will study how the chloroplasts in Elysia maintain a functional PSII repair cycle for months.

References

Acknowledgments
Academy of Finland and Finnish Cultural Foundation are acknowledged for financial support.
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 biosensing 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 [1]. This approach proved to be successful in identifying D1 mutations conferring enhanced stability, tolerance to free-radicals-associated stress and competence for herbicide perception [2]. 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 [3]. 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.

References

Acknowledgements
This research was performed within the FP7-SME-2008-1 SENSBIOSYN project (ID: 232082) and the CMST COST Action TD1102 PHOTOTECH.
Laser Induced Forward Transfer (LIFT) is a direct write technique, applied for the printing of many types of materials such as metal inks, organic materials and biomaterials. In this work, we present a new approach of using LIFT technique, for the characterization of the wetting properties of surfaces. Many techniques to probe the thermodynamic stability of such surfaces have already been employed (adhesion\textsuperscript{1}, drop impact\textsuperscript{2} experiments, etc.) but most of them are limited to the maximum pressure that can be induced (kPa). With LIFT technique, laser pulses are creating liquid jets with very high velocity up to 260 m/s and consequent liquid impact pressures up to 37 MPa respectively. Moreover, this techniques gives the ability to scan the wetting properties of a surface with very narrow and precisely controllable steps (down to 0.1 MPa) as far as the impact pressures applied. This unique characteristic, along with the capacity to print liquids of various viscosities makes this technique an ideal candidate to characterize surfaces that withstand high liquid impact pressures without losing their liquid repelling properties, as well as to outline their possible applications. The LIFT experiments were carried out, using a pulsed Nd:YAG laser (266 nm, 10 ns ) and a high power imaging micromachining system with a microscope 15x OFR objective lens. For the pump probe experiments, a second Nd:YAG laser at 532 nm was used as a probe laser, while both lasers were synchronized by a pulse delay generator. For the measurements of the jet velocities, pump probe experiments were carried out and will be presented, revealing the laser printing mechanisms, as well as the pinning and/or the droplet roll off from the surface. The pinning threshold was evaluated by side view contact angle measurements setup. The time resolved images reveal the liquid impact dynamics and the roll off and/or pinning of the droplets on the surface, defining the pressure value above which the surface is no longer liquid repellent. For liquid impact pressures, above the pinning pressure threshold, contact angle measurements are presented for droplets with 100-150 μm diameter, and 2 nL volume, pinned at on surfaces with variable various topographies. For pressure values slightly just above the pinning threshold, high contact angle is observed while further increase of the impact pressures leads to further penetration of the droplets on the morphology of the surfaces and thus lower contact angles are recorded.

References

Acknowledgements
The authors kindly acknowledge funding from the Hellenic and European Regional Development Funds (ERDF) under the Hellenic National Strategic Reference Framework (NSRF) 2007–2013, of the Project “THALIS-DESIGN and fabrication of Robust supErHyDROphobic/philic surfaces and their application in the realization of ‘smart’ microfluidic valves” and by the European Union (European Social Fund—ESF), the Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF)—Research Funding Program: Heracleitus II investing in knowledge society through the European Social Fund and the European Research Council under the European Community’s Seventh Framework Program (FP7/2007–2013) COST Action TD1102 Photosynthetic proteins for technological applications: biosensors and biochips (PHOTOTECH) and the funding from the European Union (European Social Fund—ESF).
Much research efforts are focused on implementation of sensor applications exploiting photosynthetic bio-recognition elements mostly due to the high susceptibility of the primary photosynthetic reactions towards variety of chemical species [1]. Photosynthetic microalgae are often among the preferred sensing elements in biosensor devices for large-scale and in field monitoring of different classes of pesticides or heavy metals. The current challenges in using photosynthetic bio-recognition element are mainly related to improving the stability and sensitivity of the biological component.

The ultimate goal of this research is the realization of robust, sensitive, and reliable photosynthetic sensing elements by altering the aminoacid composition of the D1 protein of photosystem II (PSII) in the model algae *Chlamydomonas reinhardtii*. Taking advantage of protein engineering techniques and computational analyses it was possible to identify a set of single point mutations in D1 conferring improved tolerance to free-radicals-associated stress and/or competence for herbicide perception [2, 3].

Particularly, this study is focused on photochemical characterisation of *Chlamydomonas* strains hosting a substitution of D1-Ile163 with Asn (I163N) and D1-Phe265 with Thr (F256T) or Ser (F265S) by registering thermoluminescence (TL) glow curves and kinetics of oxygen-evolving reactions. Both methods provide comprehensive information about the efficiency of the PSII photochemistry and offer useful criteria for the functional characterisation step of the bio-recognition element. The results indicated that the mutation introduced near to the PSII redox-active Tyr_{161} (I163N) may stabilize PSII charge separation, while the alteration in the composition of the Q_{B} binding pocket (F256T and F265S) lead to noticeable impairment of the Q_{A} to Q_{B} electron transfer. Peculiarities of the PSII performance in selected strains and their suitability as bio-recognition elements will be presented.

References


Acknowledgements

The authors acknowledge Grant by COST Action TD1102 PHOTOTECH.
P10 - DIURNAL CHANGES OF PHOTOSYNTHESIS IN ARTHROSPIRA CULTURES GROWN IN A THIN-LAYER SLOPING PLATFORM AND AN OPEN POND

K. RANGLOVÁ1, J.R.F. MALAPASCUA1,2, A.M. SILVA-BENAVIDES3,4, G. TORZILLO5, J. MASOJÍDEK1,2
1 Laboratory of Algal Biotechnology, Inst. of Microbiology, Academy of Science, Opatovický mlýn, Třeboň, Czech Republic
2 Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
3 Escuela de Biologia, Universidad de Costa Rica, San Pedro, San José
4 Centro de Investigacion en Ciencias del Mar y Limonologia (CIMAR), Universidad de Costa Rica, San José, Costa Rica
5 National Research Council, Inst. per lo Studio degli Ecosistemi, Sesto Fiorentino, Italy

The microalga A. (Spirulina) platensis is one of production strains grown in large-scale mass cultures as food and feed supplement [1]. Various unfavourable conditions can affect the growth. Alterations of cultivation conditions are reflected in changes of photosynthetic activity that can be used to estimate culture growth and productivity [2]. Generally, Chl fluorescence technique (fast induction kinetics and pulse-amplitude modulation) is a powerful tool for testing photosynthetic activity of outdoor microalgae cultures in order to estimate growth and productivity [3]. The aim of these experiments was to study the acclimation processes in cultures of A. platensis, strain M2 grown outdoors in open systems: a mixed circular pond (biomass density of 0.5 g L⁻¹; 10 cm deep; total area of 1 m²) and a thin-layer sloping platform (biomass density of 5 g L⁻¹; 0.7 cm deep; total surface of 5 m²; biomass density of 5 g L⁻¹). The cultures were grown under optimal (35°C) and suboptimal (25°C) temperatures at ISE-CNR, Sesto Fiorentino, Italy during high summer (August). Changes in photosynthetic activity and physiological status were monitored by two techniques—fast fluorescence induction kinetics (AquaPen AP-100, P.S.I. Brno) and saturation pulse analysis of fluorescence quenching (PAM-2500, H. Walz) that can reflect the immediate photosynthetic performance of a culture. In model trials, we monitored diurnal changes in the maximum photochemical yield of PSII (Fv/Fm) and the relative electron transport rate (rETR) calculated from rapid light-response curves measured by pulse-amplitude modulation technique. At optimal temperatures (35°C) mean values of the Fv/Fm ratio (maximum photochemical yield of PSII) were found 0.6 in the sloping platform and 0.56 in the open pond, while rETR (maximum electron transport rate) value was found 153 in the platform and 96 (-40%) in the pond. These changes may be attributed to the significant difference in the surface-to-volume ratio (one order of magnitude higher in the platform) and to scarce turbulence achieved in the pond. In the cultures grown at suboptimal temperatures (25°C), the mean value of the Fv/Fm ratio was 0.57 in the cascade and 0.53 in the open pond, while maximum rETR value was found 134 in the platform and 103 (-23%) in the pond. In the platform-grown cultures, fast fluorescence induction kinetics showed a significant increase in the 2-ms inflection J (the accumulation of the reduced “primary” acceptor of PSII, QA) as well as 30-50 ms inflection I (the reduction of the QA and PQ pool) at 25°C as compared with the cultures grown at 35°C that indicated stress. In the thin-layer platforms the cultures grown at optimum temperature (35°C) showed by about 20% higher photosynthetic activity (rETR) as compared to those grown at suboptimum temperature (25°C) while in the open pond there was much less difference in photosynthesis between the cultures grown at 35 and 25°C. We demonstrate here that cultivation of A. platensis in the thin-layer platform is feasible, and productivity attained can be by about 25% higher than that obtained in the pond, both at optimal and suboptimal temperatures. Lower productivity in the pond is explained by the fact that in this system the cultures tend to be acclimated to low light.

References

Acknowledgements
This study was supported by the Ministry of Education, Youth and Sports, projects Algatech CZ.1.05/2.1.00/03.0110, Algain CZ.1.07/2.3.00/30.0059 and Algatech Plus LO1416 'Centre for Algal Biotechnology'.
The possible application of biological materials in technical developments (e.g., environmentally friendly biodegradable items, bioelectronic and biosensor devices) is in the focus of large number of studies in research of academic and industrial laboratories because bio-composites (among them bio-nanocomposite materials) are considered to be the materials for future [1]. Biological systems offer inherently good examples for phenomena (note that the size of most cell organelles are micrometers or sub-micrometers, the thickness of the biological membranes and protein complexes are ca. 5-10 nm) and also for their use in applications in nanotechnology [2]. Light sensitive biomolecules, as well as photosynthetic reaction center complexes are promising as the biological components because of their extremely fast performance and efficient energy conversion. Fabrication of systems for efficient light energy conversion (e.g., photovoltaics), integrated optoelectronic systems or biosensors (e.g., for specific detection of pesticides) can be visualized for the near future [3-4]. A couple of examples of the applications of the photosynthetic reaction centers purified from purple bacteria in nanotechnology will be presented here. Measuring light induced change in current in electrochemical cell (called photocurrent) is an elegant demonstration of the suitability of the photosynthetic systems for photovoltaics, or other practical applications in optoelectronics (e.g. for sensing elements for specific compounds, like pesticides). Two of our RC based composites were successfully tried as active (working) electrode applications in electrochemical cells. It has been demonstrated that continuous redox turnover of nanocomposite prepared from PTAA/MWCNT and RCs bound to ITO can be driven by light if quinone is added to the solution for mediating the electron transport between the working and the counter electrodes. The induced photocurrent can be inhibited by specific inhibitor, like terbutryn. The study of possibility for generating photocurrent in dried organic solar cell sensitized by RC protein is also under progress and preliminary results will be presented.

References

Acknowledgements
Work was supported by grants from the Swiss Contribution (SH/7/2/20), the Hungarian OTKA (K112688 and PD116739). One of the authors (E.Ny) acknowledges the financial support of Stephan W. Kuffler Research Fellowship. Thanks are due to the helpful discussions with members of PHOTOTECH COST TD1102.
The marine environment is continuously challenged by the input of about 200,000 types of chemical compounds, mostly through human and industrial activities. These chemicals enter the marine environment via atmospheric deposition, because of runoff, direct discharges of wastes and wastewaters from land into the sea, through 'in-situ' production by aquaculture or through accidents and illegal discharges from ships. Recent reports emphasize that future monitoring strategies should largely embrace techniques based on biological effects as opposed to chemical analysis only, which is considered insufficient since it does not provide any evidence of whether or how chemicals affect ecosystems. Here we report on miniaturized algal photosystem assays, that integrate whole living unicellular microalgae in a microdevice with optical illumination and detector elements, as a sensor for both early warning and first-line screening of toxic compounds in marine water. This system provides a reliable, cost-effective and sensitive detection for substances that interfere with either photosynthesis or cellular metabolism [1]. The algal cells are small in size and assays using photosystem II fluorescence can be downscaled and parallelized in micro-engineered fluidics units with integrated detectors. An additional advantage of the algal sensors is that photosystem II activity can be monitored both by optical and electrochemical transduction systems that reduce the risks of false positive signals. However, hypo- and hyper-saline conditions affect many sites involved in photosynthesis process and bring about significant changes in the photosynthetic performance of individual algae from analyte and influence biosensor response. Therefore, it is important to select appropriate strains to improve stability and adaptability of the microalgae into a water source in which they will be used. In order to select the suitable microalgae for marine monitoring, algae strains from different taxonomic groups were tested. Experiments were carried out to understand behavior and sensitivity of free algae strains to high salinity conditions in the presence and absence of target molecules. Results showed that except for Nannochloropsis (Eustigmatophyceae), which has high growth rate and chlorophyll content, none of the other strains, like Scrippsella sp from the Dinoflagellate family or those of the Diatom family responded well to interesting analytes due to their poor growth rate and low chlorophyll content. Non-marine microalgae C. reinhardtii strain IL (an intronless mutant of the wild-type) and its mutants obtained by mutagenesis of the photosynthetic D1 protein or irradiation with UV light (called antiox and UV mutants respectively, Johanningmeier et al. unpublished) are substantially affected in presence of seawater, masking only in part the effect of biocide presence. In addition, Chlorella genus from this taxonomic group able to establish symbiosis with protozoa, has also been shown good sensitivity and tolerance. Many reports have suggested that lipids could be involved in the protection against salt stress, telephone we studied the lipid content of the tested microalgae and its relationship in biosensing marine water. Finally, integration of free and immobilized algae into microchips with different size and shape, in lab-on-a chip design, is reported.

References

Acknowledgments
This work is supported by Photosynchlamy ESA project, Bravoo EU Call for Ocean of tomorrow and Cost Action Photoch.
In the complex path of the oxygen evolution in the Photosystem II the crucial step resides in the transition from the S2 to the S3 state of the Kok-Joliot's cycle, in which the high mobility of the atoms in the cluster could be one of the reason of its high efficiency. On the ground of our previous computational chemistry calculations on Photosystem II models, which show an inversion of stability between the S2B+ and S2A+ state [1-2], we propose a reorganization mechanism involving an hydroxyl (W2) and a -oxo bridge (O5) which is able to link the closed cubane S2B intermediate conformer to the S3 open cubane final structure [3]. This mechanism can reconcile the apparent conflict between recently reported water exchange and EPR experiments, and theoretical studies [4-5].

References
Efficient light harvesting in photosynthesis is achieved by pigment-pigment excitonic interactions, which depend on the molecular architecture of the pigment-protein complexes. Extraction of the plant light-harvesting complex II (LHCII) from the native membrane or from aggregates by the use of surfactants brings about significant changes in the excitonic circular dichroism (CD). To elucidate the cause of these changes, e.g. trimer-trimer contacts or surfactant induced structural perturbations, we compared the CD spectra of LHCII aggregates, artificial and native LHCII-lipid membranes and LHCII solubilized in detergent or trapped in polymer gels. By this means we identified CD signatures of LHCII-LHCII interactions and interactions with the detergent and lipids. Furthermore, we used anisotropic CD (ACD) of macroscopically-aligned LHCII, a novel approach in photosynthesis, to uncover the orientation of the excitonic transitions with respect to the protein geometry. The changes in pigment-pigment excitonic interactions in LHCII, brought about by its molecular environment, alter the flow of excitation energy. Depending on the environment, LHCII showed a different distribution of excited-state lifetimes, measured by time-resolved fluorescence. We examined the exciton relaxation and equilibration dynamics in isolated LHCII trimers, aggregates, and reconstituted LHCII:lipid membranes by using two-dimensional electronic spectroscopy (2DES). Correlating the frequencies of coupled exciton states, ultrafast transient 2DES is a powerful technique to resolve the excited-state dynamics in complex multichromophore systems such as LHCII. We were able to resolve different pathways of exciton relaxation occurring on time scales from hundreds of femtoseconds to several picoseconds. The relaxation of specific intermediate-energy Chl a exciton states was accelerated in quenched LHCII aggregates compared to trimers or reconstituted membranes. The results shed new light on the energy transfer and excitation quenching dynamics relevant to the processes of light harvesting and photoprotection in natural photosynthesis.

Acknowledgements
This work was supported by grants from the Hungarian National Research Fund This work was supported by the Hungarian Scientific Research Fund (OTKA-PD 104530, TAMOP-4.2.2.A-11/1/KOV-2012-0060), Hungarian National Innovation Office and A*STAR Singapore (NIH-A*STAR TET_10-1-2011-0279).
AUTHORS INDEX
- A -

ACAR-SOYKUT Esra 25
ACIKBAS H. Sebnem 25
AGARWAL Vivechana 37
AGOSTIANO Angela 39
AKHTAR Parveen 54, 72
ALLAHVERDIYEVA-RINNE Yagut 20
ANTAL Taras 51
ANTONACCI Amina 22, 40, 65, 67
ANTONUCCI Alessandra 33
ARTERO Vincent 13

parvidua@gmail.com
allahve@utu.fi
taras_an@mail.ru
amina.antonacci@ic.cnr.it
vincent.artero@cea.fr

- B -

BALINT Ioan 40
BARANOWSKY Michal 24
BARHUM Hani 59
BELVISO Danilo 39
BERSANINI Luca 20
BERTALAN Ivo 22, 65, 67, 70
BIALEK Rafał 49, 60
BIRTA Balaz 69
BOVI Daniele 71
BOYACI Ismail Hakki 25
BRAUN Artur 30
BURZAN Niels 30

hani.barhum@mail.huji.ac.il
Rafal.bialek@gmail.com
ihb@hacettepe.edu.tr
Artur.Braun@empa.ch

- C -

CADIRCI Bilge Hilal 45
CAFFARRI Stefano 17, 61
CALIANDRO Rocco 39
CAMPI Gaetano 22, 34
CAPONE Matteo 71
CARMELI Chanoch 31, 59
CARMELI Itai 31, 59
CHANAewe Alina 29
CHATZIPETROU Marianneza 38, 56, 66
CHIRIZZI Daniela 38, 63
CORRIE Alexander S. 36
COSKUN Halime 33
CONZUELO Felipe 28
CREPIN Aurelie 61
CROCE Roberta 18
CSEKO Richard 69

bilgehilal.cadirci@gop.edu.tr
stefano.caффarri@univ-amu.fr
gaetano.campi@ic.cnr.it
cpnmtn@gmail.com
ccarmeli@post.tau.ac.il
mchatzip@central.ntua.gr
crepinaurelie@hotmail.fr
r.croce@vu.nl
AUTHORS INDEX

- D -
DEAK Zsuzsanna 12
DELGADO Juan David 29, 46  j.d.delgadodiaz@vu.nl
DENARO Renata 70
DI BARTOLO Natalie 36  natalie.dibartolo@bristol.ac.uk
DINC Emine 18
DOROGI Marta 50, 54, 72
DUBAS Katarzyna 24

- E -
ELLINAS Kosmas 56
ENRIQUEZ Miriam M. 54, 72
EREMIA Sandra A.V. 40
ERMAKOVA Maria 20

- F -
FACCIO Greta 30
FARINOLA Gianluca M. 33, 39
FARKA Dominik 33
FEIFEL Sven C. 47
FEILKE Kathleen 19
FRESE Raoul N. 29, 46  r.n.frese@vu.nl
FRIEBE Vincent M. 29, 46  v.m.friebe@vu.nl

- G -
GARAB Gyozo 50, 54, 72  garab.gyozo@gmail.com
GERGELY Csilla 37
GIACOBBE Maria Grazia 70
GIARDI Maria Teresa 22, 35, 65, 70  mariateresa.giardi@mlib.ic.cnr.it
GIBASIEWICZ Krzysztof 24, 49, 60, 62  krzyszgj@amu.edu.pl
GIERA Wojciech 62  w_giera@amu.edu.pl
GIOTTA Livia 38, 63  livia.giotta@unisalento.it
GLOWACKI Eric Daniel 33
GOGOLIDES Evangelos 56
GOLUB Maksym 14
GRABCHEV Ivo 32  grabchev@mail.bg
GRUBER J. Michael 29
GUALCIDI Maria Rachele 38, 63  maria.rachele.guascito@unisalento.it
GUIDONI Leonardo 21, 71  l.guidoni@gmail.com

- H -
HAJDU Kata 37  hajdu.kata@gmail.com
HARNIMAN Rob 36
HARPER F.M. William 36
HARTMANN Volker 28
HAVURINNE Vesa 64 vetahav@utu.fi
HEIFLER Omri 31
HEJAZI Mahdi 47
HOWE J. Christopher 20
HUOCCO Tuomas 20

- I -
IBRAHIM Mohamed 14
ITALIANO Francesca 39, 63

- J -
JANSSEN J.D. Paul 30, 41 pjanssen@sckcen.be
JOHANNINGMEIER Udo 22, 65, 67, 70
JONES Mike R. 24, 29, 36, 49 m.r.jones@bristol.ac.uk

- K -
KANBUR Yasin 33
KLEM Karel 26, 55 klem.k@czechglobe.cz
KRIEGER-Lizskay Anja 19 anja.krieger-liszkay@cea.fr
KROLL Alexandra 30
KUJAWA Melania 49 melania.kujawa@op.pl
KUKARSKA Galina P. 51
KUMAR Karuppannan Senthil 31

- L -
LAMBREV Petar H. 50, 54, 72 lambrev.petar@brc.mta.hu
LAMBREVA Maya D. 22, 34, 40, 65, 67 maya.lambreva@ic.cnr.it
LEA-SMITH David J. 20
LEYS Natalie 30, 41
LIGUORI Nicoletta 18
LISDAT Fred 47
LITESCU Simona C. 40 slitescu@gmail.com
LOKSTEIN Heiko 14, 47

- M -
MAGYAR Melinda 50, 69 magyarmelu@gmail.com
MAHAINI Talal Al 41
MALAPASCUA Jose R.F. 27, 68
MALITESTA Cosimino 63
MARQUEZ Jessica 37
MASLENKOVA Liliana T. 67
MASOJIDEK Jiří 27, 68 masojidekJ@seznam.cz
AUTHORS INDEX

MASSAOUTI Maria 38, 66 mmassaouti@iesl.forth.gr
MASTROGIACOMO Ludovico Valli 63
MATTILA Heta 55
MATTOO Autar 11 Autar.Mattoo@ARS.USDA.GOV;
MAZUREK Wojciech 62
MENTES Ozay 25
MERSCH Dirk 48
MEZZETTI Alberto 23 alberto.mezzetti@libero.it
MILANO Francesco 33, 38, 39, 66 f.milano@ba.ipcf.cnr.it
MILLO Diego 29, 46
MISHRA Anamika 55 mishra.a@czechglobe.cz
MISHRA Kumud Bandhu 55 mishra.k@czechglobe.cz
MORO Laura 70
MUN Bongjin S. 30

- N -
NAAMAN Ron 31
NAGY László 37, 50, 69 lnagy@sol.cc.u-szeged.hu
NARZI Daniele 71 daniele.narzi@gmail.com
NOVOTNA Katerina 55
NOWACZYK Marc M. 28
NYERKI Emil 69

- O -
OMAR Hassan Omar 39
OPERAMOLLA Alessandra 33, 39

- P -
PALESTINO Gabriella 37
PAPA Florica 40
PAWLAK Krzysztof 72
PEEVA Violeta 67 vnp@abv.bg
PELTIER Gilles 20
PETKOV Ivan 32 IPetkov@wmail.chem.uni-sofia.bg
PEZZOTTI Gianni 70
PIEPER Jorg 14 pieper@ut.ee
PLUMERÉ Nicolas 28, 52 nicolas.plumere@rub.de
PODHORODECKI Artur 24
POLTICELLI Fabio 22, 65, 67 politicel@uniroma3.it

- R -
RADU Gabriel-Lucian 40
RAGNI Roberta 39
RANGLOVA Karolena 27, 68 ranglova@alga.cz
REA Giuseppina 22, 34, 40, 65, 67  giuseppina.rea@mlib.ic.cnr.it
REISNER Erwin 48
RELLA Simona 63
RICHAUD Pierre 20
ROGNER Mattias 28
ROY Laura M. 18
RUSSO Daniela 34  russo@ill.fr

-  S  -
SARICIFTCI Niyazi Serdar 33
SASS Laszlo 12
SCHRANTZ Krisztina 30
SCHUHMANN Wolfgang 28, 52
SCOGNAMIGLIO Viviana 22, 40, 65, 67  viviana.scognamiglio@ic.cnr.it
SERGEJEVOVA Magda 27
SILVA BENAVIDES Ana Margarita 68
SIPKA Gabor 50
STANO Pasquale 22
STAPF Stefanie 52  stefanie.stapf@rub.de
STIEGER Kai R. 47  kai.stieger@th-wildau.de
STOYANOV Stanimir 32
SWAINSBURY David 29, 36  D.Swainsbury@bristol.ac.uk
SZABO Tibor 69  tiberatosz@gmail.com
SZEWCZYK Sebastian 62

-  T  -
THAN Howe-Siang 54, 72
TANGORRA Rocco Roberto 33, 39
TIAN Lijin 18
TORZILLO Giuseppe 53, 68
TOULOUPAKIS Eletherios 53  toulou_e@chemistry.uoc.gr
TROTTA Massimo 33, 38, 39, 63  massimo.trotta@cnr.it
TSEKENIS George 66
TSEREPI Angeliki 56
TUREMIS Mehmet 70
TYYSTJÄRVI Esa 51, 55, 64  esa.tyystjarvi@utu.fi

-  V  -
VAN GRONDELLE Rienk 29
VANDENHOVE Hildegarde 41
VANHOUDT Nathalie 41
VASILESCU Ioana 40
VASS Imre 12  vass.imre@brc.mta.hu
VITEK Petr 26
AUTHORS INDEX

VON HAUFF Elizabeth L. 29
VRANDECIC Kamarniso 14

- X -
XU Pengqi 18

- Y -
YORDANOVA Stanislava 32

- Z -
ZERGIOTI Ioanna 38, 56, 66  zergioti@central.ntua.gr
ZHANG Cheng 54, 72
ZHANG Jenny 48  jz366@cam.ac.uk
ZHAO Fangyuan 28
ZIMANYI Laszlo 37
ZOBININA Veranica 22
ZOUNI Athina 14, 47