cost TD1102

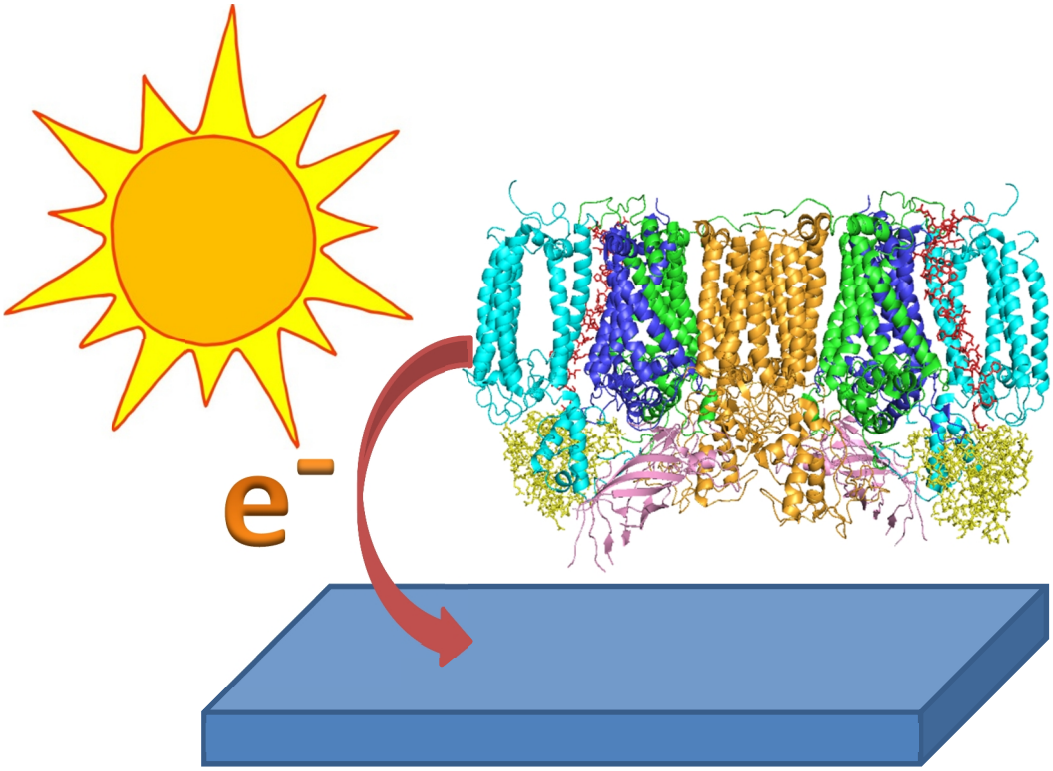
PHOTOTECH: BIOSENSORS & BIOCHIPS

SCK·CEN
STUDIECENTRUM VOOR KERNENERGIE
CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE

EUROPEAN SCIENCE FOUNDATION

First Plenary Workshop

Antwerp, Belgium, from 10 to 12 April 2013



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Status: Unclassified

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COST Action TD1102

Photosynthetic proteins for technological applications: biosensors and biochips (PHOTOTECH)

http://www.cost.eu/domains_actions/cmst/Actions/TD1102
http://www.phototech.eu/

First Plenary Workshop

Antwerp, Belgium, from 10 to 12 April 2013
http://www.sckcen.be/nl/Evenementen/PHOTOTECH2013

Venue:
Radisson Blu Astrid
Koningin Astridplein 7
2018 Antwerp, Belgium
Tel: +32 (0)3 203 1234
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Chair:

Guiseppina Rea (CNR, Italy)

Scientific Committee:

Fabio Polticelli (University of Roma, Italy)
Bao-Lian Su (University of Namur, Belgium)
Laszlo Nagy (University of Szeged, Hungary)
Esa Tyystjarvi (University of Turku, Finland)
Raoul Frese (Free University Amsterdam, The Netherlands)
Nicolas Plumeré (Ruhr University Bochum, Germany)

Local Organizer:

Paul Janssen (SCK•CEN, Belgium)

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Foreword

Photosynthesis is a paramount biological process enabling and sustaining aerobic life on Earth. Embedded into asymetrically charged lipid bilayers, individual macromolecular assemblies perform the vital light-harvesting-, charge-separation-, and water splitting functions to store solar energy in a biochemical form.

Acting at micro- and nanoscale levels as well as on a femto-picosecond timescale, macromolecular photosynthetic complexes are receiving much attention as their application allows for the construction of optoelectronic devices consisting of mono- and/or multi-molecular layers of naturally obtained or engineered subcomponents. In this era of nanotechnology, systems biology, and synthetic biology, the development of photosynthetic protein-based biosensors, biochips, and photovoltaic semiconductors will no doubt form the cornerstone towards advances in artificial biomimetics.

This meeting should provide an updated overview on the structural-functional features of the most promising photosynthetic proteins suitable for biotechnological applications, in particular biosensoring, and address other topics such as the form and immobilization of suitable biomediators and the different stages of the actual manufacturing process.

Hence, the meeting has been divided in four topics that are broadly aligned with the four Workgroups established for this COST Action. Each of these sessions are spearheaded by an outside speaker to bring in new ideas and allow for additional scientific interactions. On Friday we have the Management Committee meeting. In parallel to that, a Brainstorm is organised to stimulate all attendees in sharing their thoughts and start discussions on any topic that they seem fit, whether scientifically or on the organisation of events, the exchange of students, or the use of equipment. Halfway the MC meeting, everyone will join together for a coffee break, at which point already suggestions can be exchanged. Also, some MC members may join the Brainstorm before or after the break.

The program of 26 lectures arranged into two days, the first of which includes an evening session, followed by a Friday morning of discussions, is certainly intense and demanding. But we hope that your participation will be rewarding and that your stay in Antwerp will be a pleasant one!!

Giuseppina Rea, CNRI

Paul Janssen, SCK•CEN

EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

www.cost.eu/cmst

COST Action no. TD1102

Photosynthetic proteins for technological applications: biosensors and biochips (PHOTOTECH)

2011 | 2015

Objectives

- Sustainable European network in the field of photosynthesis-based biosensors.
- Deepen knowledge on charge separation and electron transfer phenomena in RC proteins either in the native form or interfaced with inorganic-organic materials.
- Laying a foundation for future development of bio-hybrid devices.
- Exploring the power of molecular biology and bioinformatics as tools to improve the performance of photosynthetic complexes as biomediators.
- Demonstrating the capability of keeping the proper functionality of the biomediators in a hostile environment, such as solid-state devices.
- Delivering robust, reliable, environmental-friendly and sensitive biosensor prototypes.
- Promoting EU mobility among early-stage researchers.
- Contributing to technology transfer (TT) to EU industrial networks and innovation/TT offices.

Main Achievements

- Plant Photosystem-II and bacterial Reaction Center mutants were designed in order to improve the affinity for triazine type herbicides for biosensor application and for computer simulations.
- Simplified procedures were explored for adhering purple bacterial photovoltaic complexes to bare metal electrodes for photocurrent generation, biosensing and biocomputing applications.
- Photosystem-I and specific P450 protein complexes were combined for potential applications as photo-sensors with the sensitivity of fluorescence.
- Mass spectrometric method for the measurement of singlet oxygen in plant photosynthesis was developed.
- Bacterial and plant photosynthetic reaction center proteins were immobilized in inorganic surfaces for possible applications in optoelectronics.
- An in vitro directed evolution approach was targeted to the Photosystem II to isolate biosensing elements for triazine and urea herbicides in the nanomolar range.

Chemistry and Molecular Sciences and Technologies (CMST)

Participating countries

CH, CZ, DE, DK, ES, FI, FR, HU, IL, IT, IE, PL, PT, SK, SE, TR, UK

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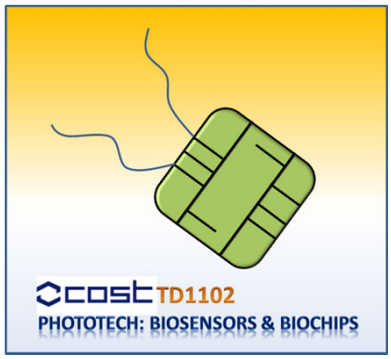

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EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

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Working Group activities

Working Group 1

BIOMEDIATOR SELECTION & ENGINEERING

- Whole cells, photosystems, reaction centers, chimerics & hybrids, artificial peptides
- Bioinformatics
- Molecular Biology

Working Group 2

BIOMEDIATOR IMMOBILIZATION

- In-situ (redox copolymer, silica matrix)
- Layer-by-Layer (LIFT)
- Dry junction (metal NP, CNT, semiconductor)

Working Group 3

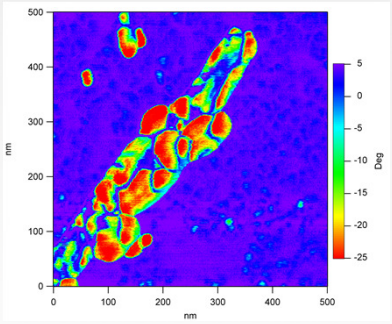
BIOSENSOR MANUFACTURE

- Optical (fluorimeter, phototransistor)
- Electrochemical (amperometer)

Working Group 4

COMPONENTS CHARACTERIZATION

- Geometry & Morphology
- Biological
- Chemical
- Physical
- Interface



Phase image of photosynthetic reaction center/carbon nanotube bio-nanocomposite

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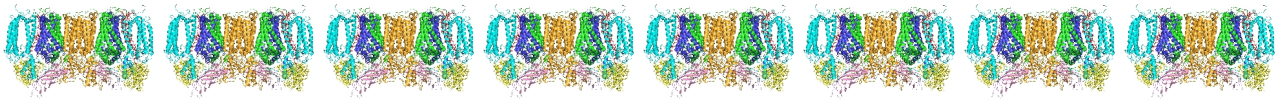
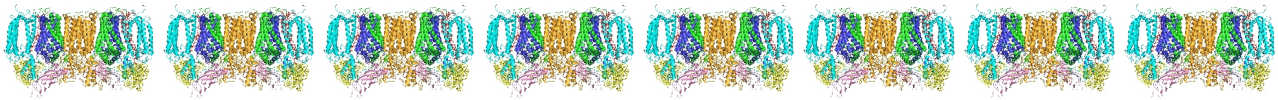


COST Action TD1102– First Plenary Workshop

Photosynthetic proteins for technological applications: Biosensors and biochips (PHOTOTECH)

Wednesday – Friday, 10 - 12 April 2013

Radisson Astrid Blu, Antwerp, Belgium



Program (more information: pjanssen@sckcen.be)

Wednesday 10 April	
13:30	**Registration** (welcome coffee served)
14:00	**Practical announcements**
14:10	OL – **Giuseppina Rea (CNR Italy)** " Photosynthesis: 2.4 bilion years later "
	Session I: Biomediator Selection (WG1)
14:40	KS1 – **Stenbjörn Styring (Uppsala Universitet, Uppsala)** " Molecular science for solar fuels - recent results in natural and artificial photosynthesis "
	Coffee break (15:25 – 15:50)
15:50	L01 – **Fabio Polticelli (Universita di Roma Tre, Rome)** " Computational studies of photosystem II. Functional insights and biotechnological applications "
16:15	L02 – **Maya Dimova Lambreva (NRCI, Rome)** " Photoelectrochemical herbicide sensing driven by *Chlamydomonas reinhardtii* bio-recognition elements "
16:40	L03 – **Viviana Scognamiglio (NRCI, Rome)** " New biomimetic peptides for herbicide detection "
17:05	L04 – **Hanene Badri (SCK•CEN, Mol)** " Photosynthesis in *Arthrospira* is not effected by acute doses of gamma radiation "
17:30	**Administration - ESF**
	Dinner (18:30 – 20:00)

Session II: Biomediator Immobilization (WG2)

20:00 KS2 – **Linda Thöny-Meyer (EMPA, St-Gallen)**
" Playing Biomimetic Puzzles on Photoelectrochemical Hematite Cells: Proteins, Pigments & Co. "

20:45 L05 – **Marianneza Chatzipetrou (NTUA, Athens)**
" A high sensitivity polyphenol amperometric biosensor realized by laser Induced Forward Transfer "

21:10 L06 – **Nicolas Plumeré (Ruhr-Universität, Bochum)**
" Redox-Active Dendrimers for Specific Binding and Electronic Contacting of Redox Proteins "

Coffee break (21:35 – 21:50)

21:50 L07 – **Krzysztof Gibasiewicz (UAM, Poznań)**
" Mechanisms of $P^{+}H_{A}^{-}$ recombination in reaction centers from *Rhodobacter sphaeroides* "

22:15 L08 – **Artur Braun (EMPA, Dübendorf)**
" Phycocyanin-hematite hybrid electrode with light antenna functionality for solar hydrogen generation by photoelectrochemical water splitting "

22:40 L09 – **Chanoch Carmeli (Tel Aviv University, Tel Aviv)**
" Properties of Hybrid PSI-Carbon Nanotubes, Metals and Semiconductors "

Thursday 11 April

Session III: Biosensor manufacture (WG3)

09:00 KS3 – **Erwin Reisner (University of Cambridge, Cambridge)**
" Photocatalytic water oxidation with photosystem II and bio-inspired hybrid materials "

09:45 L10 – **Melinda Magyar (University of Szeged, Szeged)**
" Strategies to bind photosynthetic reaction centers to nano-systems "

10:10 L11 – **Frank Müller (Rurh-Universität, Bochum)**
" Double intrachain histidine-tag for isotropic self-assembly of redox enzyme on electrode surfaces "

Coffee break (10:35 – 11:00)

11:00 L12 – **Mehmet Mutlu (Haceteppe University, Ankara)**
" Modification of Quartz Crystal Microbalance Surfaces via Electrospun Nanofibers and RF Plasma Disharge Intended for Biosensor Applications "

11:25 L13 – **Paquale Stano (Universita di Roma Tre, Rome)**
" Semi-synthetic minimal cells: from origin of life to synthetic biology"

11:50 L14 – **Kata Hajdu (University of Szeged, Szeged)**
" Carbon nanotubes quench singlet oxygen generated by photosynthetic RCs "

12:15 L15 – **Simona Carmen Litescu (NIRD Biol. Sc., Bucharest)**
" Critical Issues in Manufacturing Biosensors Based on Photosynthetic Organisms "

Lunch (12:45 – 14:00)

Session IV: Components Characterization (WG4)

14:00 KS4 – **Francesco Milano (CNR-IPCF, Bari)**
" Enhancing light harvesting capability of the photosynthetic reaction centre by a tailored molecular fluorophore "

14:45 L16 – **Esa Tyystjärvi (University of Turku, Turku)**
" The F_0 rise phenomenon reveals that 500 nm and 650 nm but also 560 nm light favors PSII over PSI "

15:10 L17 – **Violeta Peeva (IPPG, Sofia)**
" Investigation of photosynthesis in intact organisms by thermoluminescence "

Coffee break (15:35 – 16:00)

16:00 L18 – **Ivo Grabchev (Sofia University, Sofia)**
" Zn (II) and Cu (II) halide complexes of poly(propylenamine) dendrimer investigated by Infrared and Raman spectroscopy "

16:25 L19 – **Alberto Mezzetti (Université de Lille, Lille)**
" Infrared difference spectroscopy : a valuable tool in the investigation of the mechanism of photosynthetic reactions "

16:50 L20 – **Daniela Russo (CNR-IOM, Grenoble)**
" From whole cells towards photosynthetic reaction centres: dynamics properties for biotechnological applications "

17:15 L21 – **Touloupakis Eleftherios (University of Crete, Heraklion)**
" Biohydrogen production by the unicellular algae *Chlamydomonas reinhardtii* "

17:45 **Administration – ESF // Group picture**

Beer Tasting Event (18:30 – 20:00) (Bier Central, De Keyserlei nr. 25)

Friday 12 April

Parallel sessions

09:00 **(1) Magement Committee meeting**

09:00 **(2) Brainstorm**

Continuously coffee and beverages served (09:00 – 12:00)

PHOTOTECH 2013, Antwerp

Timetable - Overview

Wed 10 April		Thu 11 April		Fri 12 April	
Welcome Coffee (13:30 - 14:00)		WG3 09:00 – 10:35	KS3: E. Reisner	MC (9:00-12:00)	BS (9:00-12:00)
			L10: M. Magyar		
			L11: F. Müller		
		25 min BREAK		25 min BREAK	
		WG3 11:00 – 12:40	L12: M. Mutlu	MC	BS
			L13: P. Stano		
			L14: K. Hajdu		
			L15: S. Litescu		
		Lunch (12:45 - 14:00)		Administration + Goodbye	
WG1 14:00 – 15:25	Announcements (10 min)	WG4 14:00 – 15:35	KS4: F. Milano		
	OL : G. Rea (Chair)		L16: E. Tyystjärvi		
	KS1: S. Styring		L17: V. Peeva		
25 min BREAK		25 min BREAK			
WG1 15:50 – 17:30	L01: F. Polticelli	WG4 16:00 – 17:40	L18: I. Grabchev		
	L02: M. Lambreva		L19: A. Mezzetti		
	L03: V. Scognamiglio		L20: D. Russo		
	L04: H. Badri		L21: E. Touloupakis		
Dinner (18:30 - 20:00)		Beer Tasting (18:30 -20:00)			
WG2 20:00 – 21:35	KS2: L. Thöny-Meyer	+ group picture (17:45) + administration (17:45 - 18:15)			
	L05: M. Chatzipetrou				
	L06: N. Plumere				
15 min BREAK		free evening in Antwerp			
WG2 21:50 – 23:15	L07: K. Gibasiewicz				
	L08: A. Braun				
	L09: C. Carmeli				

OL = 30 min
KS = 35/40 + 5 min
L = 15/20 + 5 min

Opening Lecture

OL (Wed/14:10 – 14:40)

Photosynthesis: 2.4 bilion years later

Giuseppina Rea

CNR - Institute of Crystallography, Roma, Italy

E-mail: giuseppina.rea@mlib.ic.cnr.it

Bio-geochemical studies address the carbon fixation that occurred more than 3 bilion years ago by anaerobic bacteria exploiting hydrogen, or organic or sulphur compounds, as electron donor. Thereafter, oxygenic photosynthesizers arose being able to split water and release oxygen. This *oxygen revolution* eventually gave rise to a respiration-sustaining atmosphere and led to the accelerated evolution of life. Some 2.4 bilion years later, life is requiring a new revolution and once again photosynthesis could shape the future as oxygen, food, and energy are mandatory for life on Earth. Today, capturing and applying the power of photosynthesis is a great challenge in all research fields. Also, the steadily increasing loss of arable land and the continuous growth of the human population bring about agro-food strategies to improve the photosynthesis yield. So, crop breeders may one day cultivate crops engineered with bacterial antennae to broaden the absorption spectrum of the light harvesting complex, while biologists could improve other photosynthetic traits in organisms normally void of photosynthesis or even create synthetic plastid-driven endosymbioses.

Native or engineered photosynthetic systems in electronic biosensing devices will allow for a rapid and reliable assessment of food quality- and safety. And in a time of global warming, owing to atmospheric pollution by 'greenhouse gasses', photosynthetic microalgae will become increasingly important in the field of fossil fuel replacement, carbon mitigation, and bioenergy.

In terms of artificial photosynthetic mimicry, higher photocurrent densities may be achieved in dye-sensitized solar cells. In addition, efficiencies for energy conversion could be improved by novel dye assemblies. Similarly, peptide assemblies, protein complexes, protein-inorganic hybrid material, and supra-molecular proteinic structures could form the new nanomaterial for energy production. However, the structure and chemistry of native core reaction centres are not yet completely understood. Studies on the chromophore antennae of two cryptophyte genera hint at the existence of quantum coherence mechanisms occurring in biological energy transfer and transduction. Undoubtedly, parallel technological advances in 2-D echo spectroscopy and time-resolved X-ray crystallography will help us to better understand and control the processes to efficiently harness sun light and convert it into clean and carbon-neutral fuels.

On a final note, let us not forget research in space. As space is a natural harsh environment missions into space offer unique opportunities to study life and matter, including the effects of space conditions on photosynthesis. As such, exciting extra-terrestrial inspiration may await us.

Session WG1: Biomediator Selection

KS1 (Wed/14:40 – 15:25)

Molecular science for solar fuels - recent results in natural and artificial photosynthesis

Stenbjörn Styring

Department for Chemistry, the Ångström Laboratory, Uppsala University, Uppsala, Sweden

E-mail: stenbjorn.styring@kemi.uu.se

The lecture will cover visions and strategies in research in the Swedish Consortium for Artificial Photosynthesis. It will outline the need for solar fuels on a global scale and motivate various types of research in the field. Our own research aims for the production of hydrogen, from the endless resources solar energy and water. We follow two scientific branches to accomplish solar fuels, artificial photosynthesis in entirely manmade systems and photobiological solar fuels production in green algae and cyanobacteria. The lecture will cover recent results from both fields. In our attempts to accomplish artificial photosynthesis we use a photoactive Ru-center to drive water oxidizing catalysts or proton reducing catalysts. The lecture will cover our results on a Co-nanoparticle able to oxidize water. Recent EXAFS studies and their structural implications will be covered.

The lecture will also describe recent results on the redox situation in Photosystem II that is developed during sulphur deprivation in the green algae *Chlamydomonas reinhardtii*. We have combined EPR, flash induced variable fluorescence and thermoluminescence in wild type and mutants. Photosystem II is degraded during sulphur deprived conditions to between 25-50% of its original concentration dependent on which mutant is studied. The level is probably regulated by the balance between photosynthesis and respiration. It is found that a major fraction of the electrons that are channelled to hydrogen come from Photosystem II. An interesting conclusion is that the induction of hydrogenase in the cell relieves the redox pressure on Photosystem II. This opens up forward electron transfer after a nearly complete block early during sulphur deprivation.

References:

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2. Shevchenko, D., M.F. Anderlund, A.Thapper, and S. Styring, S. *Energy and Environmental Science*, **2011***, 4,* 1284-1287
3. Risch, M., D. Shevchenko, M.F. Anderlund, S. Styring, J. Heidkamp, K.M. Lange, A. Thapper, and I.J. Zaharieva.*Hydr. Research*, **2012**, 37, 8878-8888.
4. Volgusheva, A., Styring, S. and Mamedov, F. *PNAS,* **2013** (In Press).

L01 (Wed/15:50 – 16:15)

Computational studies of photosystem II. Functional insights and biotechnological applications

Fabio Polticelli

Department of Sciences, University of Roma Tre, Rome, Italy

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Photosystem II (PSII), a 350 kDa multi-subunit protein-cofactor complex located in the thylakoid membranes of oxygenic photosynthetic organisms, is an oxidoreductase whose biological function is that of catalysing the light-induced production of reducing equivalents in the form of reduced plastoquinone molecules. The byproduct of PSII activity is molecular oxygen and thus this system is essential to make life on Earth possible. Plastoquinone reduction to plastoquinol lowers PSII affinity for the latter molecule and leads to its release. However little is known about the structural and energetic bases of this process. We have used molecular dynamics simulations of the complete PSII complex embedded in a lipid bilayer to investigate the plastoquinol release mechanism. A distinct dynamic behaviour of PSII in the presence of plastoquinol is observed which, coupled to changes in charge distribution and electrostatic interactions, causes disruption of the interactions seen in the PSII-plastoquinone complex and to the "squeezing out" of plastoquinol from the binding pocket. Results obtained help to shed some light on the mechanism of plastoquinol-plastoquinone exchange supporting the recently proposed "single channel" mechanism as opposed to the "alternating" one. The same approach has also been used to study PSII interaction with the herbicide atrazine leading to the identification of amino acid positions whose mutation can improve PSII affinity for this molecule, and opening up interesting perspectives for the development of novel PSII-based biosensors. Alternative, less computationally intensive approaches, such as molecular docking simulations, will be also illustrated and their potential in the design of photosystem variants endowed with novel recognition properties discussed.

L02 (Wed/16:15 – 16:40)

Photoelectrochemical herbicide sensing driven by *Chlamydomonas reinhardtii* bio-recognition elements

Maya Lambreva[1]
& A. Antonacci[1], I. Husu[1], G. Rodio[2], V. Peeva[3], V. Scognamiglio[1], K. Buonasera[1], I. Bertalan[4], F. Polticelli[5], L. Maslenkova[3], U. Johanningmeier[4], M.-T. Giardi[1], and G. Rea[1]

[1]*Institute of Crystallography - National Research Council, Monterotondo Scalo, Rome, Italy;*
[2]*Biosensor srl, Rome, Italy;*
[3]*Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria;*
[4]*Martin-Luther-University, Plant Physiology Institute, Halle (Saale), Germany;*
[5]*Department of Biology, University Roma Tre, Rome, Italy.*

[1]*E-mail: maya.lambreva@ic.cnr.it*

Photosynthetic organisms share a natural ability to bind anthropogenic pollutants impairing distinctly detectable photochemical processes. This property allowed us to exploit whole photosynthetic cells for the construction of an electrochemical biosensor targeted to environmental monitoring. Among photosynthetic organisms sensitive to pesticide classes, the unicellular green alga *Chlamydomonas reinhardtii* revealed to be a smart bio-sensing element useful for the realization of amperometric analytical devices. As competitive inhibitors of the plastoquinone (Q_B) binding to the reaction centre D1 protein, triazine and urea-type herbicides block the photosynthetic electron transport leading to a reduction of the biosensor output current in a concentration-dependent manner. Output signals originated by the reduction of light-induced oxygen evolution, and the biosensor response was expressed as a ratio between the current intensities registered in the absence and in the presence of herbicides. In order to preserve algal photosynthetic functionality, the *Chlamydomonas* cells were entrapped in an alginate gel directly onto the surface of a screen-printed carbon nanotube working electrode.

Furthermore, in attempt to improve the scarce stability and sensitivity often accompanying the use of whole-cell-based biosensors, molecular engineering and computational approaches were exploited to improve the performance of photosynthetic complexes and their herbicide perception. Detailed characterization of the newly developed bio-recognition elements will be presented.

L03 (16:40 – 17:05)

New biomimetic peptides for herbicide detection

Viviana Scognamiglio[1]
& A. Antonacci[1], M. Lambreva[1], K. Buonasera[1], P. Stano[2], F. Polticelli[2], [1]G. Pochetti, M.-T. Giardi[1], and G. Rea[1]

[1]*Institute of Crystallography - National Research Council Monterotondo, Rome, Italy;*
[2]*Department of Biology, University Roma Tre, 00146 Rome, Italy*

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Exhaustive understanding of functional and structural properties of a molecule is mandatory when involved in biotechnological application. In this work we present the first efforts to create a synthetic Q_B binding pocket for herbicides detection, bioinspired on the D1 protein from photosystem II (PSII) of *Chlamydomonas reinhardtii*. By computational modelling and automated protein synthesis, the D1 plastoquinone/atrazine binding niche, in native and mutated forms, was reconstituted and the structural and functional features deeply analysed by circular dichroism, fluorescence spectroscopy and microcalorimetry. The implemented molecules showed ability to mimic the D1 protein in terms of its structure and function, and capability to bind atrazine in the nanomolar range. The application of these bioinspired molecules range from simulating a biological processes by means of minimal models, to the development of novel nanosensor array platforms with designed and controlled functions. Indeed, further aims will be focused on the set-up of an array of synthetic molecules showing a wide range of affinity towards different classes of target analytes, and on the design of different immobilisation strategies, for the development of optical biosensors for herbicides detection.

L04 (Wed/17:05 – 17:30)

Photosynthesis in *Arthrospira* is not effected by acute doses of gamma radiation

Hanène Badri[1,2]
& P. Janssen[1], N. Leys[1], R. Wattiez[2]

[1]*Molecular and Cellular Biology, Belgian Nuclear Research Center SCK•CEN, Mol Belgium;*
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The edible cyanobacterium *Arthrospira sp.* PCC 8005 was selected by the European Space Agency (ESA) as part of the life support system MELiSSA for recycling oxygen, water, and food in future long-haul space missions. However, during such extended missions, *Arthrospira sp.* PCC 8005 will be exposed to continuous artificial illumination and harmful cosmic radiation, and its high nutritive value and efficient oxygen production by photosynthesis might be effected.

After acute exposure to high doses of ^{60}Co gamma radiation, the morphological analysis of *Arthrospira* showed that, up to 5000 Gy, there were no significant effects on the overall morphology or length of filaments. Although filament fragmentation was observed at the highest dose of gamma radiation tested (6400 Gy), filaments could restart photoautotrophic growth and proliferate normally. The photosystem activity, measured as the PSII quantum yield immediately after irradiation, decreased significantly at radiation doses above 3200 Gy while through analysis of pigment content a significant decrease in phycocyanin was observed at the higest doses of 5000 Gy and 6400 Gy. This was confirmed by morphological analysis of *Arthrospira* sp. PCC 8005, showing a loss of its phycocyanin pigment after 5000 Gy. The phycocyanin pigment might act as a potent antioxidant and radical scavenger particularly in the dose range of 200-3200 Gy while this protecting activity is much less at the higher dose range.

Based on chromosomal DNA integrity assessed by agarose gel electrophoresis immediately after exposure, little or no impact of irradiation on DNA was found over the entire dose range. This was confirmed by ERIC-PCR profiling, with no changes in the DNA banding patterns between irradiated and non-irradiated cells. Using ICP Mass Spectrometry, we noted a relatively high molar ratio of Mn to Fe. As in other radiotolerant organisms, a surplus of Mn^{2+} may safeguard DNA repair proteins from oxidative inactivation and hence bolster DNA repair mechanisms.

We now have started proteomic and transcriptomic studies on *Arthrospira* sp. PCC 8005 i.e. before and after exposure to high doses of gamma radiation. This way we aim to (1) better understand the radiation tolerance in this versatile organism and (2) shed light on its ability to grow photosynthetically during large-dose exposures. Both properties are unique assets for its oxygenic growth in space and it will be highly interesting, also from a fundamental point of view, to see which proteins and enzymes are involved in the underlying processes.

Session WG2: Biomediator Immobilization

KS2 (Wed/20:00 – 20:45)

Playing Biomimetic Puzzles on Photoelectrochemical Hematite Cells: Proteins, Pigments & Co.

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The use of hematite in combination with phycocyanin, a protein of the antenna complex of photosynthetic microorganisms, has been reported to lead to higher photocurrent with long term stability in photoelectrochemical cells (PEC) [1]. This finding has raised several interesting questions with respect to how this biomimetic system works and what approaches for further improvement can be envisaged. The type of protein and pigment, the way of immobilization on the hematite film, and the reaction conditions all play an important role for optimization of the BioPEC. Protein immobilization is a crucial step when stable, long-living functions are conferred to materials. In the case of phycobiliproteins the function is provided by the cofactor, a linear protein that is kept in a specific conformation by the polypeptide. It is difficult to assess whether or not this conformation is retained when the protein is immobilized on the hematite surface. The integrity of the protein was assessed in a parallel experiment where a bacterial laccase was immobilized on hematite and tested for activity.

A biochemical approach was taken by using another natural pigment, melanin, as an additional component in the system. Melanin absorbs light over the entire range of the visible spectrum and thus might make the light harvesting more efficient. The biopolymer was produced enzymatically in situ, resulting in increased current density on the phycocyanin loaded hematite electrode. The system was further investigated by replacing the phycocyanin complex isolated from the natural *Spirulina* organism by a genetically engineered, single subunit of the phycobilisome, which now opens another dimension of tools for playing the BioPEC game.

L05 (Wed/20:45 – 21:10)

A high sensitivity polyphenol amperometric biosensor realized by laser Induced Forward Transfer

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This work presents an amperometric biosensor with high sensitivity towards phenolic compounds using laccase as biorecognition element and commercial Screen Printed electrodes. Polyphenolic compounds, such as catechol, are a class of molecules found largely in fruits and several fruit product (wines, olive oils etc). One of the enzymes widely used for the detection of such phenolic compound is laccase, since laccase is able to oxidize a broad range of substrates such as polyphenols and anilines.

For the immobilization of the enzyme, on the SPE, Laser Induced Forward Transfer (LIFT), an innovative technique has been used. LIFT is an advanced tool for the immobilization of biomaterials, since there is no need for any type of chemical functionalization layer. LIFT technique relies on the displacement of the material to be deposited from a donor substrate to a receiver substrate. The donor substrate consists of a quartz layer (1 inch), a sacrificial metallic layer (40 nm Ti) and the liquid biomaterial. LIFT enables the physical absorption of the biomaterials, on a rough surface. This mechanism is based on the high transfer velocity, of the biomaterials, which after laser irradiation impact the substrate with high pressure, leading to a complete wetting state.
Laccase solutions were prepared by dissolving a suitable amount of lyophilized enzyme with known enzymatic activity, in phosphate buffered saline (PBS) solution pH 4.5. Graphite SPEs DRP110 were used for these experiments, purchased by DropSens, which consist of a counter, a reference and a working electrode (4mm diameter). The working electrode was printed with the enzyme (32mU) in PBS via LIFT technique and afterwards, 50 µL PBS were added for amperometric measurements.

The immobilized laccase was characterized towards catechol in solution. The biosensor sensitivity was found to be 150 nM/ 50 nA for catechol, a detection limit much improved that the one recorded at the literature.

L06 (Wed/21:10 – 21:35)

Redox-Active Dendrimers for Specific Binding and Electronic Contacting of Redox Proteins.

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Fast electron transfer is a key issue in electrochemical applications of redox proteins. The present work focuses on a novel strategy toward efficient electrical communication between enzymes and electrodes based on redox-active dendrimers as bridging units. Electron relays bound to the dendrimers surface facilitate the charge transfer via electron hopping. Functional groups on the dendrimers also allow for both oriented binding of the redox enzyme and self-assembly on the electrode surface. The enzyme is a genetically engineered ferredoxin $NADP^+$ reductase (FNR) with several cysteine residues introduced by site directed mutagenesis in close proximity to the FAD cofactor. Bioconjugation techniques using these thiol groups were exploited for the coupling of the FNR to the dendrimers. High catalytic activity for NADPH oxidation was demonstrated for an FNR connected to a gold electrode via a viologen modified dendrimer bridge.

Acknowledgement: financial support by the EU and the state NRW in the frame work of the HighTech-NRW programme is gratefully acknowledged.

L07 (Wed/21:50 – 22:15)

Mechanisms of $P^+H_A^-$ recombination in reaction centers from *Rhodobacter sphaeroides*

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Absorption of light by purple bacterial photosynthetic reaction centers is followed by ultrafast, ~3-ps, charge separation between primary electron donor, bacteriochlorophyll dimer P, and electron acceptor, bacteriopheophytin H_A, leading to formation of the charge separated state $P^+H_A^-$. Normally, electron on H_A^- is transferred further to next electron acceptors. However, electron from H_A^- may hop also back to P^+ in the reaction called charge recombination. In recent years our group explored different pathways of this charge recombination reaction. It is known, that $P^+H_A^-$ may recombine either to the ground singlet state PH_A or to the triplet state ^3PH_A. The singlet ground state may be reached either directly or via the thermally activated pathways including transient formation of the state $P^+B_A^-$, B_A being an intermediate electron acceptor, bacteriochlorophyll, located half-way between P and H_A. $P^+H_A^-$ recombination is multiexponential process, and the multiexpoenentiality of this reaction was shown to be related to the dynamic response of the protein surrounding the electron transfer cofactors. Modulation of intraprotein electron transfer by the dynamic properties of the protein has to be taken into account when trying to employ reaction centers into biohybrid devices. Extraction of the dynamic parameters of protein reacting to charge separation and modulating $P^+H_A^-$ charge recombination will be in the focus of the talk.

L08 (Wed/22:15 – 22:40)

Phycocyanin-hematite hybrid electrode with light antenna functionality for solar hydrogen generation by photoelectrochemical water splitting

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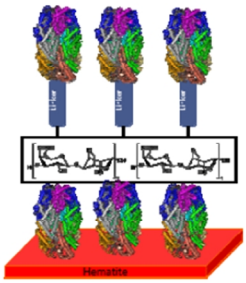
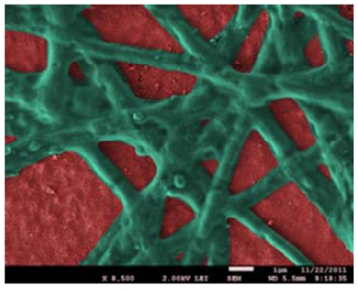
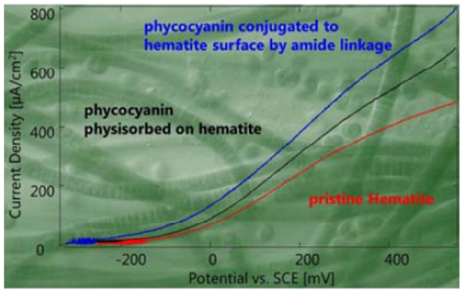
Artificial photosynthesis (AP) and photovoltaics (PV) have developed independently as scientific and technological disciplines. PV has emerged as a readily available sustainable energy technology with a steadily increasing market share. AP is increasingly taking center stage in scientific interest, but progress towards a viable technology is still missing. Solar hydrogen generation by water splitting over semiconducting metal oxide electrodes in photoelectrochmical cells (PEC) is a resurging field of scientific and increasingly also technological interest. We demonstrate how the covalent attachment of light harvesting antenna protein c-phycocyanin from blue green algae on iron oxide photoanodes yields a substantial increase in water splitting photocurrent and hydrogen evolution. We will also show how further refinement of the synthesis, processing and conjugation of motifs from cells and proteins further enhance the device efficiency.







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L09 (Wed/22:40 – 23:15)

Properties of Hybrid PSI-Carbon Nanotubes, Metals and Semiconductors

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Efficient electronic junctions were fabricated by oriented covalent binding of genetically engineered cysteine mutants of photosynthetic reaction center protein photosystem I (PS I) to solid surfaces. PS I is a protein-chlorophyll complex that converts visible light to photopovoltage of 1 V in 200 ns with quantum efficiency of 100% and absorbed light energy conversion efficiency of 47%. Hybrid PS I-solids generated under dry environment photocurrent at a turnover rat of 15 ns, injected photoelectron to semiconductor surface at ps and greatly enhanced the conductance of carbon nanotubes. These properties make the hybrid PS I-solids good candidate for PS I based photovoltaic and optoelectronic devices.

Session WG3: Biosensor Manufacture

KS3 (Thu/09:00 – 09:45)

Photocatalytic water oxidation with photosystem II and bio-inspired hybrid materials

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The sunlight-driven splitting of water into H_2 and O_2 represents a sustainable route for the production of the energy vector, H_2. We have recently made some progress in the integration of enzymes and synthetic catalysts in metal oxides for electro- and photocatalytic H_2 generation,[1-3] but a fuel forming reductive process such as H_2 evolution can only operate in a sustainable redox cycle if electrons are provided from an oxidative process such as water oxidation to O_2. Water oxidation is generally considered as the major challenge in water splitting and this reaction will be the focus of this presentation. Nature' s water oxidising enzyme, photosystem II (PSII), sets a benchmark in terms of O_2 evolution rates under ambient conditions and serves as an inspiration to develop synthetic water oxidation photocatalysts. We adsorbed PSII from *Thermosynechococcus elongatus* on a nanostructured and transparent metal oxide electrode for visible light driven water oxidation to O_2 (Figure 1)[4]. The three-dimensional and hydrophilic metal oxide surface supports photoactive PSII films, promotes high protein loadings and allows for direct electron transfer at the enzyme-electrode interface.

We have also developed a straightforward method to assemble a purely synthetic, bio-inspired water oxidation photocatalyst based on a metal oxide nanocomposite system (unpublished results). The PSII-based and synthetic systems will be compared and limitations and prospects of these artificial photosynthetic hybrid materials for photocatalytic water oxidation discussed.

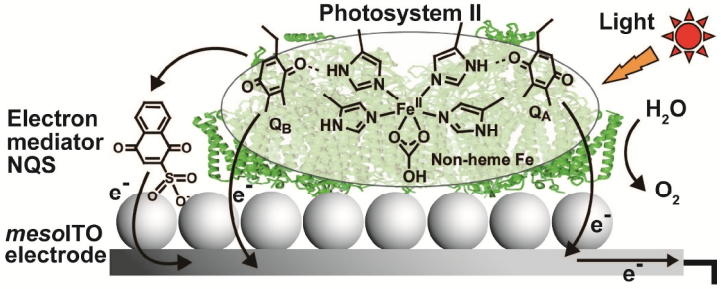

Figure 1.: Schematic representation of visible light driven water oxidation with photosystem II integrated in a nanostructured indium tin oxide (ITO) electrode.[4]

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NOTES

L10 (Thu/09:45 – 10:10)

Strategies to bind photosynthetic reaction centers to nano-systems

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It is known that biological materials are developed by the evolution for extremely efficient and specific functions in a very high sensitivity. However, losing the original (in vivo) environment their operability usually rapidly decreases. The idea to find the proper carrier in order to save their unique efficiency could be revolutionary at many fields. Recently biological components, as well as photosynthetic reaction centers (RCs) were successfully integrated with nanomaterials combining their advantageous properties in new systems called bio-nanocomposites. These new types of hybrid materials open possible directions for new generations of practical applications, e.g. energy conversion and storage, integrated optoelectronics in analytical, memory and micro imaging devices, etc.

The RCs have many advantages that can be used in nano-devices: a) the main absorption change appears in the near infrared; b) there are kinetic components of the intraprotein electron transport from ps to several seconds; c) the quantum yield of the primary charge separation is almost 100% (only small part of this can be reached by Si-based materials); d) it can be in redox-equilibrium with the surrounding molecules so that light generated charges can be captured by the environment. The aim of the talk is to introduce the work of our research group on designing, creating and characterising bio-nanocomposite materials based on bacterial RC proteins and different redox active carrier matrices.

RCs are bound to different types of carbon nanotubes (functionalized, non-functionalized, single-walled, and multi-walled) by different methods (physical or chemical binding methods) and to non-carbon materials, like transitional metal oxides (ITO), porous silicon, or conducting polymers. The activity of the complexes is measured by kinetic spectroscopy (light induced absorption change, electric (conductivity measurement) or spectroelectrochemistry methods.

In our research works we are focusing on (1) finding the most convenient and efficient energy converting systems, i.e., what is the best hybrid system for this purpose, (2) the reproducibility, i.e. biological systems have a larger variability as compared to the inorganic ones, and (3) the stability of the complexes. Besides the investigation of the direct photochemical/physical processes (charge separation and stabilization processes in the RCs or those are connected directly to the photosynthetic cycle) additional (harmful or supporting) reactions are also worthwhile to investigate. Recently we started to investigate the conditions for the production of singlet oxygen accompanying the photochemistry in our composites. Based on our experiences we try to create and investigate other redox proteins (e.g., hydrogen peroxidase) in different nanosystems.

L11 (Thu/10:10 – 10:35)

Double intrachain histidine-tag for isotropic self-assembly of redox enzyme on electrode surfaces

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In biosensing and biofuel cell applications, control of enzyme orientation on surfaces is crucial to ensure accessibility to the active site and efficient electrochemical communication between the electrode's surface and the enzyme's redox site. Affinity binding between a terminal histidine-tag and an electrode surface functionalized with metal complexes allows for oriented immobilization of fully active enzyme monolayers (1-3). However the applications of this approaches are limited since the possible location of the terminal His-tag on the protein limits the choices in orientation.

This issue may be solved by using intrachain histidine residues (4,5) from the surface of the enzyme. However, in comparison to the terminal his-tag, the stability of the binding is significantly lower. We circumvent this issue by introducing a double intrachain histidine-tag as binding site at the protein. Based on SPR investigations we demonstrate the increased binding affinity of protein bearing two sets of His-X_3-His (two histidine separated by two amino acids) in alpha helixes on NTA-Ni(II) modified surfaces. We demonstrate that the single his-tagged protein dissociated from the NTA-Ni(II) surface via both metal-histidine dissociation and metal ion transfer processes. The presence of two binding sites in double his-tagged protein on the other hand prevents both dissociation pathways and thus yields stable protein monolayers. The binding and electrocatalytic activity of a ferredoxin $NADP^+$ reductase with the double intrachain his-tag on glassy carbon electrodes modified with NTA-Zn(II) and NTA-Cu(II) will be given as example.

Acknowledgement: Financial support by the EU and the state NRW in the frame work of the HighTech-NRW programme is gratefully acknowledged.

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L12 (Thu/11:00 – 11:25)

Modification of Quartz Crystal Microbalance Surfaces via Electrospun Nanofibers and RF Plasma Disharge Intended for Biosensor Applications

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The major aim of this study is to increase the performance of mass sensitive biosensor surfaces by making modifications on quartz crystal microbalance (QCM) surfaces via electrospinning and plasma polymerization techniques. Polyvinyl alcohol (PVA) nanofibers with a diameter of approximately 150 nm were collected on the QCM surfaces by electrospinning technique. Allylamine monomer was used to create specific groups on these nanofiber coated surfaces by plasma polymerization technique. Modified surfaces were characterized by contact angle measurements, scanning electron microscopy, atomic force microscopy, fourier transform infrared spectroscopy and QCM frequency measurements in order to determine the physical and chemical characteristics of the surfaces after each experimental stage. N-H, C-N, C-H and C=O group bands were determined in the IR spectra of the materials. Decrease in the contact angle values of the modified materials indicated the increase in hydrophilicity. Those results showed that amine containing films on the surfaces were successfully deposited using plasma.

The performance of modified QCM surfaces was tested via resonance frequency shifts measurements after bovine serum albumin immobilization. In this group of tests, “dip and dry” method and “flow-cell method” were performed and 548±4 Hz and 50±5Hz frequency shifts were obtained respectively. Results of this study revealed that plasma treated electrospun PVA nanofiber modified surfaces can be used for further biosensor applications.

L13 (Thu/11:25 – 11:50)

Semi-synthetic minimal cells: from origin of life to synthetic biology

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In recent years, we have proposed the concept of semi-synthetic minimal cells (SSMCs), Luisi et al., 2006; Stano et al., 2011). These are cell-like compartments, based on lipid vesicles (liposomes), filled with the minimal number of biochemical species in order to display living-like properties, like self-maintenance and self-reproduction, or to perform functions for biotechnological purposes.
Born within the origin-of-life research, SSMCs are now an important pillar of synthetic biology (SB).

In SB, the technology of SSMCs aims at constructing simple cell-like systems from molecular components, according to the requirements of the minimal genome. Also in this case, one important goal is the generation of functional cell-like systems that are able to perform living-like functions. This "bottom-up" approach is different from the alternative "top-down" approach, typical of most of SB studies based on modification of living organisms. In this respect, SSMCs research avoids bioethical issues related to SB. The current state-of-the-art focuses on the production of proteins within such synthetic systems.

Our increasing ability of assembling compartmentalized systems is rapidly growing, and paves the way to interesting biotechnological applications. One of this is the construction of novel vehicles for the delivery of drugs, proteins, nucleic acids to cells. We have recently formalized – but not yet realized - a new approach where SSMCs are seen as a tool for biochemical-ICT (information and communication technology), where SSMCs are allowed to establish, possibly, two-ways chemical communication with living cells (Stano et al., 2012).
In this contribution, we will shortly introduce the concept of SSMCs, give an essential review on the state-of-the-art of current studies, with particular attention to the work carried out in our laboratory, and present some future perspectives for their possible applications, especially for realizing new tools for nanomedicine, drug delivery, and bio-ICT.

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L14 (Thu/11:50 – 12:15)

Carbon nanotubes quench singlet oxygen generated by photosynthetic RCs

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Photosensitizers may covert light into formation of reactive oxygen species (ROS) including, e.g., singlet oxygen ($^1O_2$), superoxide anion (O_2^-), and hydroxyl radicals ($^{\cdot}OH$), chemicals with extremely high cyto- and potential genotoxicity. Photodynamic ROS reactions are determinative in medical photodynamic therapy (cancer treatment with externally added photosensitizers) and in reactions damaging the photosynthetic apparatus of plants (via native pigments). The primary events of photosynthesis take place in the chlorophyll containing reaction center protein complex (RC), where the energy of light is converted into chemical potential. ROS are formed by both bacterial and plant RCs in high light and if the quenching of $^1O_2$ is impaired (1-3). In plant physiology, reducing the formation of the ROS and thus lessening photooxidative membrane damage (including the RC protein itself) and increasing the efficiency of the photochemical energy conversion is of special interest. Carbon nanotubes, in artificial systems, are also known to react with singlet oxygen (4).

To investigate the possibility of quenching of $^1O_2$ by carbon nanotubes in a biological system, we studied the effect of carbon nanotubes on $^1O_2$ photogenerated by photosynthestic RCs. 1,3-diphenylisobenzofuran (DPBF), a dye responding to oxidation by $^1O_2$ with absorption change was used to measure $^1O_2$ concentrations. $^1O_2$ was produced by excitation of either a photosensitizing dye (methylene blue) or the photosynthetic RCs in the presence of carbon nanotubes. Our results indicate that the $^1O_2$-induced absorption change of DPBF at 420 nm decreased in the presence of carbon nanotubes, suggesting that carbon nanotubes are potential quenchers of this ROS *in vivo*, too. We also tested the possibility to use tetramethyliperidine to detect the $^1O_2$ generated by the RC preparations but mass spectrometric determination of the N-oxyl reaction product was unsuccessful.

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L15 (Thu/12:15 – 12:40)

Critical Issues in Manufacturing Biosensors Based on Photosynthetic Organisms

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The present work deals with a short overview on the critical issues arising when biosensors based on photosynthetic organisms have to be manufactured.

The influence of solid supports characteristics, functionalization protocols and immobilization techniques on biosensors performance characteristics in terms of sensitivity and robustness is discussed with respect to transduction type.

Carbon- based and silica- based materials are considered in the context of model compounds immobilization and photosynthetic organisms immobilization.

Study case for Si-NWFET PSII sensors is presented, drawbacks and advantages of using this technology being emphasized.

Session WG4: Components Characterization

KS4 (Thu/14:00 – 14:45)

Enhancing light harvesting capability of the photosynthetic reaction centre by a tailored molecular fluorophore

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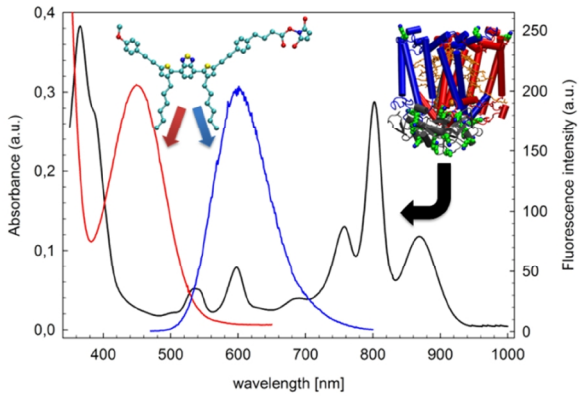
A photosynthetic reaction center (RC) is the nature's solar battery, converting light energy into chemical potential in the photosynthetic membrane, thereby assuring carbon reduction in cells. Although RC functions on the nanometer scale, with nanoscopic power, this is the protein that assures the energy input practically for the whole biosphere on Earth. The extremely large quantum yield of the primary charge separation (close to 100%) in the RC has pushed huge efforts to use it in artificial light harvesting systems. However, the its main absorption peaks are in the UV (below 350 nm) or in the NIR (above 700 nm) and there are regions (400-500 and 650-700 nm) where RC absorption is very weak (black line in the figure). In this work we propose the concept of tailored organic fluorophores as antenna covalently bound to the proteic scaffolding of the RC, capable of extending the useful range of wavelengths for energy photoconversion.

In particular, the tested fluorophore (AE) belongs to the class of of aryleneethynylenes and has been designed and synthesized to fulfill the spectroscopic, chemical, and steric requirements to act as antenna for the RC. The conjugated backbone of AE absorbs light at 450 nm (where the RC absorption is at a minimum) and has a large Stokes shift with an emission maximum at 602 nm, which corresponds to an RC absorption peak.



The succinimidyl ester group in AE enables selective covalent binding of the fluorophore to the Lys of the RC. The presented data show that the RC can be made fully photoactive at 450 nm through a fluorescence resonance energy transfer between the organic antenna and the pigments of the protein, without altering its the energetics and ability to drive the photocycle.

L16 (Thu/14:45 – 15:10)

The F_0 rise phenomenon reveals that 500 nm and 650 nm but also 560 nm light favors PSII over PSI

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After switching off illumination of a leaf, chlorophyll *a* fluorescence yield first drops but then fluorescence transiently increases and then decreases in about two minutes. The postillumination bump in the fluorescence curve is called the F_0 rise and it was earlier explained by assuming that NADPH and reduced ferredoxin accumulate in the light and reduce the plastoquinone pool which equilibrates with the Q_A electron acceptor in the dark. We tested the hypothesis about reduction of Q_A by reduced plastoquinones. For this, Arabidopsis leaves were illuminated for 8 min with moderate light and after switching the light off, the measuring beam of the PAM fluorometer was chopped by switching it on only for 0.5 s every 5 s. No F_0 rise was found to occur, indicating that the F_0 rise requires an actinic effect of the measuring beam. Thus, F_0 rise depends on reduction of Q_A by the measuring beam. The weak light of the measuring beam causes a rise in fluorescence because reduction of plastoquinone by NADPH and/or ferredoxin (cyclic electron flow) produces plastohydroquinone which binds to the Q_B site and acts as an inhibitor of electron flow. We also saw that preillumination with specific wavelengths is needed to prime the chloroplasts to a condition in which F_0 rise can be induced.

Next, the actinic effect of the measuring beam was replaced with a controllable system by using a chopped measuring beam and simultaneously applying continuous, low-intensity light ("postillumination") (PPFD 0.5 to 2.5 µmol $m^{-2}s^{-1}$) on top of it. Systematic action spectroscopy showed that preillumination at 520, 630 or 690 nm followed by postillumination at 500, 560 or 650 nm produces a large F_0 rise, and no F_0 rise occurs if the wavelengths are switched (e.g. 560 nm light is used for preillumination and 520 nm for postillumination). Furthermore, 520, 630 or 690 nm illumination was found to oxidize P_{700} and cause dephosphorylation of LHCII, while 500, 560 or 650 nm light caused phosphorylation of LHCII and did not oxidize P_{700}. These data show that F_0 rise occurs only if preillumination with moderate light favoring PSI is followed by illumination with low light favoring PSII. Furthermore, the data show that the relative action spectrum of PSII over PSI has a peak at 560 nm light whereas 520 and 630 nm favor PSI over PSII.

L17 (Thu/15:10 – 15:35)

Investigation of photosynthesis in intact organisms by thermoluminescence

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Thermoluminescence technique which consists of cooling the photosynthetic sample before or immediately after illumination, followed by progressive warming reveals PSII charge pairs recombination as well resolved thermoluminescence bands. Various treatments or stress factors may affect thermoluminescence emission compared to control by modifying the shape and/or intensity of existing bands (mainly variation in B bands), inducing new bands (Q band, C band), thus revealing the alteration of PSII electron transfer activity. Aferglow (AG) thermoluminescence emission from intact photosynthetic samples excited by far-red light is generated when an electron flow induced by warming reduce Q_B to form $S_{2/3}Q_B^-$ luminescence emitting centers and peaks usually at 46 ^oC at 0.5 $^oC/s$ warming rate. Its downshift to B band means that cyclic pathways are already activated before warming.

In vivo thermoluminescence provides a tool to examine the photosynthetic metabolism changes in whole leaves or alga cells. It provides information about stroma assimilatory potential by relative intensity of the AG band after xenon flashes and activation of cyclic pathways, with Q_B operating as "sensor" for incoming electrons, etc. Examples are given of activation of cyclic electron pathways around PSI by different treatments of barley leaves, and how they are involved in the afterglow emission: the temperature downshift of the far-red induced AG band, corresponding to an increased rate of the overall reduction of $P700^+$; increased AG band intensity, indicative for strong assimilatory NADPH+ATP potential in slowly dehydrated barley leaves.

L18 (Thu/16:00 – 16:25)

Zn (II) and Cu (II) halide complexes of poly(propylenamine) dendrimer investigated by Infrared and Raman spectroscopy

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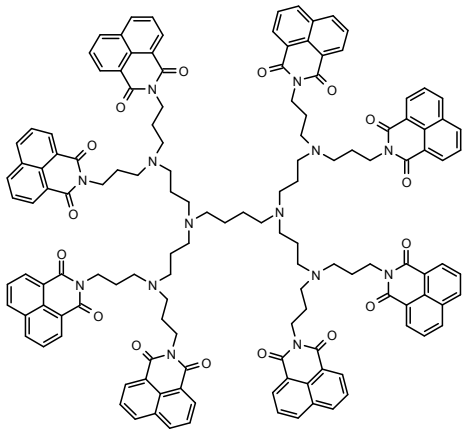

Poly(propylenamine)s are a new class of dendrimers possessing tertiary amino groups in the core and terminal primary amino groups in their periphery. Modification of the periphery with versatile fluorophores allows customizing the dendrimers functional characteristics. In response to the needs of vanguard sensors for preventing environment pollution we have been studying extensively the dendrimers modification with 1,8-naphthalimides

In this study poly(propylenamine) dendrimers whose periphery has been modified with photoactive 1,8-naphthalimide units and their metal complexes with Cu^{2+} at Zn^{2+} ions were characterize by two nondestructive and complementary spectral methods - Infrared and Raman spectroscopy. The characteristic spectral bands of the dendrimer metal complexes have been interpreted and attributed to clarify the nature of complexes formed.
These initial studies of dendrimer metal complexes are the starting point of our subsequent studies on dendrimers modified with peptides and artificial antenna systems for sensor utilizations.

Acknowledgment: The authors would like to thank the ESF for support via COST TD1102

L19 (Thu/16:25 – 16:50)

Infrared difference spectroscopy : a valuable tool in the investigation of the mechanism of photosynthetic reactions

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Infrared spectroscopy is a well-established technique for structural determination of molecules and biosystems since the beginning of molecular biology. In the last two or three decades, a modification of this technique, called infrared difference spectroscopy, has become increasingly popular for the investigation of the mechanism of biochemical reactions [1]. The development of time-resolved infrared difference spectroscopy has allowed to go a step further, enabling direct, real-time monitoring of biochemical reactions. Compared to other techniques (e.g. laser flash photolysis) this technique makes it possible to monitor basically all the molecular groups of the system under investigation (cofactors, amino acid side chains, polypeptide side chains, lipids…). Information to the atomic level can be obtained, so that phenomena like the protonation of the side chain of a given amino acid or the displacement of a cofactor can be followed. The technique has been widely used to study photosynthetic reaction centers (RCs) and light harvesting (LHs) systems, providing key results to understand the mechanism of several processes, e.g. the coupling between proton and electron transfer [2, 3 and refs therein], pigment-protein interactions [4], photoprotection events [5,6,7], or the interaction between herbidices and the Q_B binding pocket in photosystem II RC [8]. Some reactions have been followed in intact membranes [9] or even in living microorganisms [10].

I will briefly describe the principles of the technique and show some examples of applications in photosynthesis. I will then describe the results obtained in my lab on photosynthetic RCs (notably the RC from *Rb. sphaeroides*) and LHs. Some perspectives will also be discussed.

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L20 (Thu/16:50 – 17:15)
From whole cells towards photosynthetic reaction centres: dynamics properties for biotechnological applications.

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Photosynthesis gain renewed interest due to the possibility to integrate whole plant cells or their photosynthetic sub-components into optoelectronic devices such as biosensors for environmental monitoring. In this context, it is of great relevance to study the function/dynamics relationships of genetically modified photosynthetic organisms, in order to identify the parameters underlying an increased performance in terms of charge separation, protein stability and functional reliability. 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 bio-sensors. 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. Some of these mutants displayed improved sensitivity and selectivity for different classes of herbicides. Results show that point genetic mutations may notably affect not only the biochemical proterties but also the T dependence of the whole complex dynamics describing a wild type system always more rigid than the les performant mutants. In addition, a complementary hydration water collective dynamics investigation reveal with a distinct sound propagation speed not only a more rigid structure of hydration water than intracellular water but also of the native compare to the mutatant. Our results suggest a new direction of investigation and improvement of engeneering bio-sensor.

L21 (Thu/17:15 – 17:40)

Biohydrogen production by the unicellular algae *Chlamydomonas reinhardtii*

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Unicellular green algae have the ability to operate in two distinctly different environments (aerobic and anaerobic) and to photosynthetically generate molecular hydrogen (H_2). A recently developed metabolic protocol permitted separation of photosynthetic O_2 evolution and carbon accumulation from anaerobic consumption of cellular metabolites and concomitant photosynthetic H_2 evolution in the light. The H_2-evolving metabolic process was induced upon sulfate nutrient deprivation of the algae, which reversibly inhibits photosystem-II and O_2-evolution in their chloroplast. In the absence of O_2, and in order to generate ATP, green algae resorted to anaerobic photosynthetic metabolism, evolved H_2 gas in the light and consumed endogenous substrate. Hydrogen generation in green algae draws attention to an alternative cellular metabolism and offers a new avenue of research in photosynthesis.

PHOTOTECH: Photosynthetic proteins for technological applications: biosensors and biochips

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CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE

Microbiology

Context

Bacteria are single-cell organisms and play a key role in our daily live and the environment. Bacteria are amazingly abundant (ca. 10^{29} cells on Earth) and are extraordinarily diverse. They are key actors in diverse processes, including the good digestion of food in our body, the biogeochemical cycle in soils and the oxygen production in the oceans. They also play a role in food production as fermenters, industrial applications as catalysts, or can be a hazard in conservation protocols as spoilage or deteriorating agents, and in health care as cause of infectious diseases.

Objectives

The research unit Microbiology studies the behaviour of bacteria in 'extreme' environments, i.e. environments where they are exposed to toxic (radioactive) metals or ionising radiation, such as heavily polluted soils, nuclear installations and waste disposal sites or outer space. With the acquired knowledge, we want to better prevent bacterial activity when harmful, or use bacteria for our benefit via biotechnology, on Earth and in space. We study the bacterial behaviour at the multi-cellular, cellular and molecular level, using a diversity of modern high quality cellular and molecular analysis tools and data analysis through bioinformatics.

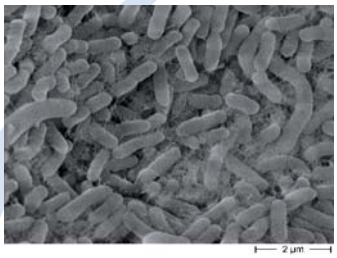
Main activities

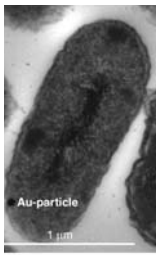
We provide research, services and training on:

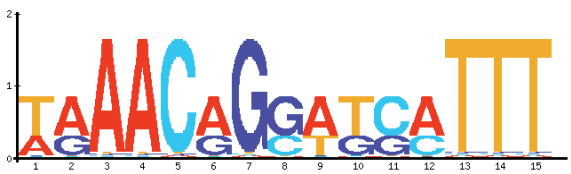
Bacterial interaction with (radioactive) metals and bacterial radiation resistance

Molecular analysis of microbes for a better understanding of:

- Bacterial detoxification systems for (radioactive) metals:
  - To determine the fate of metals in the environment.
    e.g. detection of toxic metals via biosensors/ biomarkers.
    e.g. biodetoxification and/or -extraction of toxic pollutants.
    e.g. gold bioprecipitation from mine tailings.
  - To enable better or novel (re)use of metals.
    e.g. use of silver as biocide in drinking water.
    e.g. production of biogenic metal nanoparticles.
- Molecular mechanisms providing radioresistance in bacteria, to facilitate radioprotection or health beneficial applications.





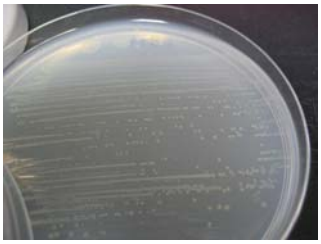


***Investigation of metal-resistant bacterium* Cupriavidus metallidurans *CH34*.**
Left: Microscopy image of bacterial biofilm.
Right: Bacterial cell containing a gold particle in cell envelope.
Below: Motif in DNA sequence regulating gene expression upon exposure to metals, detected with bioinformatics.

Microbial activity in nuclear installations and nuclear waste disposal facilities

Determination of microbial presence and activity in deep geological nuclear waste repositories (e.g in clay):

- To reveal the unique microbial diversity hidden deep underground in ancient rocks and clay.
- To prevent disturbance or damage of instrumentation by microbes.
  e.g. inhibition of methane bioproduction or biofouling.
- To assess the impact of microbes on waste storage safety.
  e.g. biocorrosion by sulphate reducing microbes.
  e.g. gas production by nitrate reducing and methane producing microbes.



Investigating microbial activity in the HADES underground research laboratory at -225 m below the SCK•CEN site.
Left: Drilling for clay samples.
Above right: Cultivation of boom clay bacteria.
Below right: Characterization and identification of bacteria.

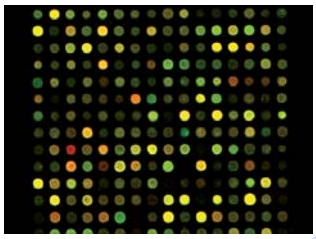
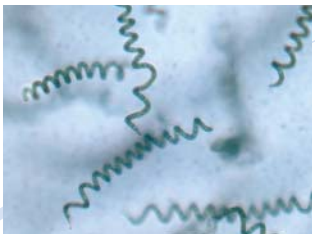
Oxygen, water and food production by bacteria in space for human space exploration

Microbial analysis for the development of a miniaturized and fully controllable artificial ecosystem called 'MELiSSA' enabling water and waste recycling and oxygen and food production with bacteria in space.

- Investigating the effects of space conditions on bacteria, including cosmic radiation and gravity.
- Selection, cultivation and characterization of photosynthetic bacteria.
- Deciphering of the DNA code and properties of edible bacteria for oxygen and food production.
- Assessment of genetic and physiological changes of bacteria over long-term cultivation in bioreactors and in space.

Research performed in collaboration with the European Space Agency ESA and a team of highly qualified European scientists.



Investigating the effects of space flight related environmental conditions on photosynthetic bacteria evolution.
Above left: Cultivation of bacteria in the International Space Station.
Above right: Cultivation of bacteria in photobioreactors.
Below left: Analysis of gene expression and regulation of bacteria grown in space.
Below right: Characterization of edible microalgea (cyanobacterium Arthrospira*) on the cellular and molecular level.*

Microbial contamination in confined space capsules and possible impact on astronaut health and spacecraft integrity

Microbial analysis for the development of biocontamination prevention, detection and mitigation protocols, to guarantee the crew biosafety during long-duration space missions in confined spacecraft.

- Characterization of bacterial contaminants found in air, water and on surfaces from spacecraft (e.g. the International Space Station) and analogues on Earth (e.g. Concordia station on Antarctica).
- Characterization of microbial dispersion and survival in ultraclean man-made (space)habitats.
- Characterization of microbial genetic rearrangements and gene transfer, on Earth and in space (e.g. with cosmic radiation).

Research performed in collaboration with the European Space Agency ESA and international space agencies.




Investigating the microbial community during the closed period.
Above left: Hibernation and isolation in the Concordia station.
Above right: Sampling the air inside the Concordia station.
Below left: Storage of samples on Antarctica in an ice cave at -50 °C.
Below right: Analysis of the samples at SCK•CEN in Belgium.

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Radiobiology

Context

Multidisciplinary radiobiological research forms the scientific basis of various disciplines such as radiation protection, radiotherapy and nuclear medicine. Consequently, radiobiology contributes to human health. The goal of radiobiological research in radiation protection is to better understand the effects of the radiation exposure at the cellular and molecular levels in order to determine the effects on health. The purpose of the research carried out in the unit of radiobiology is, for both national and international projects, to provide scientific knowledge to the authorities and to inform the population appropriately about the effects of ionizing radiation under normal or accidental circumstances.

Objectives

The main research lines that are currently studied are:

- The individual susceptibility to ionizing radiation and the related cancer risks.
- The impact of ionizing radiation on female gametes, on the development of the embryo and the brain.
- The impact of space conditions (in particular of cosmic radiation and weightlessness) on health.
- The biological effects of the medical use of ionizing radiation and radioactive substances in radiotherapy and medical imaging.

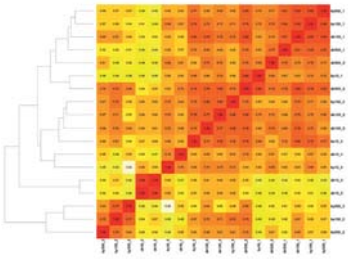
Main activities

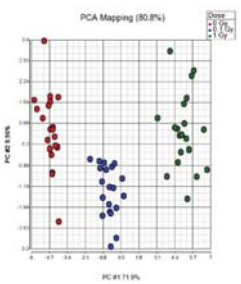
Individual susceptibility to ionising radiation and related cancer risks

This research is performed in order to:

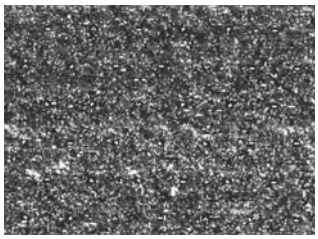
- Better understand the molecular mechanisms underlying the effects of radiation.
- Study whether low doses can induce cancerous diseases.
- Determine to which extent genetic factors can modulate cancer risk at low doses.

This research into molecular markers is also currently used for biodosimetry purposes (for example: paediatric patients subjected to computed tomography for medical imaging or biomonitoring of the population in case of a nuclear accident).





Bioinformatics analysis of irradiation effect.



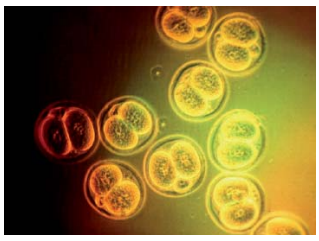
Whole genome analysis (microarrays)

Impact of ionizing radiation on the female gametes and the development of the embryo and the brain

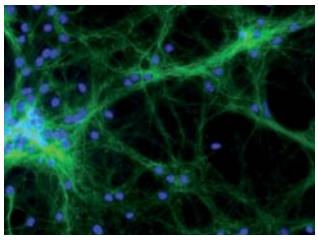
During early pregnancy, female patients can be exposed to ionizing radiation through diagnostic procedures, while being not aware of pregnancy. The potential risks of such exposures for the embryo partially depend on its genetic constitution (figures above).

Mice with various genetic backgrounds are used as models to predict the potential effects of ionizing radiation at the levels of prenatal death, congenital malformations or growth retardation, as well as their underlying molecular mechanisms.

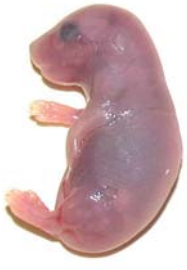
Cognitive effects induced by low and moderate doses of radiation as observed amongst the survivors of Hiroshima and Nagasaki is an important issue in radiation protection. The radiosensitivity of the brain at early stages of embryogenesis and the mechanisms of radiation-induced mental retardation are studied using a combination of animal models, in vitro cell culture and molecular techniques.



2-cell mouse embryos



Brain cells

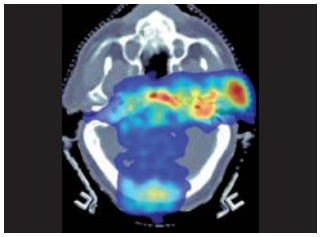


Mouse fetus

Insight into cancer treatment and cancer risk prediction

Hadrontherapy

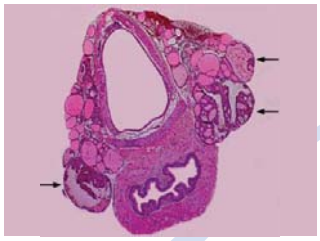
Hadrontherapy, or particle therapy, is a relatively new form of external radiotherapy, where the patient is irradiated with charged particles (protons or carbon ions, instead of X-rays). We conduct research on both the biological and physical aspects related to carbon therapy. On one side we study the response of different cell models, to carbon ions. On the other hand, we are characterizing dose response, in particle fields, for several kinds of dosimeters.



Brain tumor irradiation by hadrontherapy.

Defining the genetic component of thyroid cancer risk at low doses

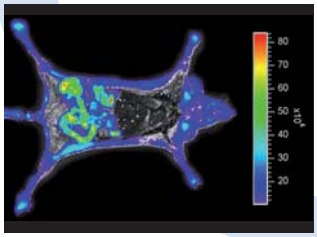
Thyroid cancer is one of the malignancies that can be induced following radiation exposure as supported by epidemiological studies of different radiation-exposed groups such as: the survivors of atomic bombings in Japan, Marshall Islanders exposed to nuclear test fall out and children undergoing head or neck radiotherapy or accidentally exposed to radiation like after the Chernobyl accident. However, no accurate model of the dose response curve for thyroid cancer at low doses exists up till present. Therefore, we currently investigate the genetic component influencing the risk of developing thyroid cancer after exposure to low dose radiation.



Thyroid (cancer shown by the arrows) is situated beside the trachea and the oesophagus.

Cancer targeting molecules attached to radionuclides: SCK•CEN's first steps into targeted radiotherapy

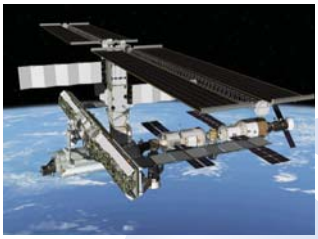
During the last decades, an increasing need for new radiopharmaceuticals has been formulated by the medical world. One of the most important applications of this type of molecules is cancer therapy. At the moment, we are studying the effectiveness of a potential radiopharmaceutical agent derived from camelidae antibodies armed with a beta-gamma emitter, Lu-177 (lutetium-177).



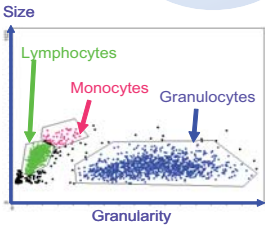
Imaging of a radiopharmaceutical for cancer treatment in a mouse.

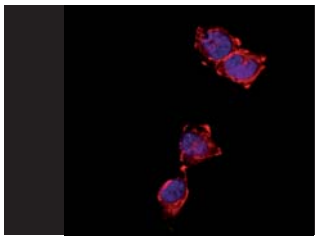
Research in space biology: how can space affect astronaut's health?

During space travel, astronauts are exposed to cosmic radiation emanating from the sun and the galaxy. The received dose can be up to 100 to 200 times the amount received on the ground for an equivalent period. The contribution from the ionizing cosmic radiation during space travel is therefore not negligible. The biological effects of space conditions on man are very complex and still insufficiently known. Continued research is therefore essential for forecasting long space flights. For this reason, cellular, biochemical as well as genetic and epigenetic changes induced in cells or astronauts residing in the international space station (ISS) are investigated by modern and high throughput technologies. At SCK•CEN, simulated microgravity as well as radiation are particularly studied at the in vitro level. Furthermore, space analogue platforms on Earth (Concordia, bed rest and Mars 500) are also currently studied.



Left: ISS, international research facility (400 km in orbit around the Earth). Right: Concordia Station (Antarctica) used as a space-simulated platform.





Left: Blood monitoring of astronauts. Right: Cell staining to investigate space-induced effects.

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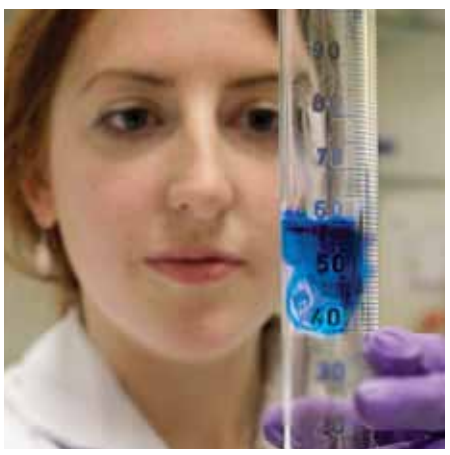

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