cost TD1102

PHOTOTECH: BIOSENSORS & BIOCHIPS

Training School on "Phototech for Biosensors and Energy",

Athens, 21-25 October 2013, Amarilia Hotel

Organised by National Technical University of Athens

Sponsored by COST action Phototech TD 1102

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I. Invited Speakers

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II. Schedule

Monday October 21st : *Welcome Reception & Round Table*

19:00 – 20:00	**Reception**	
20:00 – 21:00	**Round Table**	**Introduction-Discussion**
21:00-21:30	**Cost Project description**	**Giuseppina Rea (invited)**

Tuesday October 22nd: *Energy production, photosynthesis based photovoltaics & biomediators selection*

9:00-11:00	"Leaf-like materials capable of solar energy convention by photosynthesis"	**Bao Lian Su (invited)**
11:00-11:30 Coffee break		
11:30-13:30	"Molecular biotechnologies improving the bioreceptorial properties of Photosystem II"	**Giuseppina Rea (invited)**
13:30-15:30 Lunch Break		
15:30-17:30	"Biosensors based on aptamers detection"	**Giorgos Tsekenis (invited)**
17:30- 17:50	"Bio-photovoltaics based on hybrid systems of reaction centers and diamond"	**Roberta Caterino**
17:50-18:10	"Construction of photovoltaic cells based on Rhodobacter sphaeroides reaction centers"	**Rafal Białek**
18:10-18:30	"Screening of electricity producing profile of various photosynthetic microorganisms."	**Bilge Hilal Cadirci**

Wednesday October 23rd: *Biosensor manufacture*

9:00-11:00	"Introduction and overview of biosensors"	**Ismael Hakki (invited)**
11:00-11:30 Coffee break		
11:30-13:30	"Photosynthesis based biosensor"	**E. Touloupakis (invited)**
13:30-15:30 Lunch Break		
15:30-16:30	"Monolithic silicon interferometric optoelectronic platform for label-free multi-analyte biosensing applications"	**Ioannis Raptis (invited)**
16:30-16:50	"Photocurrent generated by photosynthetic reaction center/carbon nanotube/ito bio-nanocomposite"	**Tibor Szabó**
16:50-17:10	"A new thiol-coated interface for the development of an aptasensor for lysozyme"	**Iuliana Mihai**
17:10-17:30	"Challenges in the development of an electrochemical (bio)sensor for allergen proteins detection"	**Alis Vezeanu**
17:30-18:30 Poster Session		

Thursday October 24th: *Biosensors characterisation*

9:00-11:00	"Characterising biosensors and biosolar cells as photovoltaic devices"	**Raoul Frese (invited)**
11:00-11:30 Coffee break		
11:30-13:30	"Electron transfer in biophotoelectrochemical devices"	**Nicolas Plumere (invited)**
13:30-15:30 Lunch Break		

15:30-15:50	"Full automation of a rapid screening test for early warning measurement of phytotoxicity in water samples based on photosynthetic algae"	**Annalisa Tortelli**
15:50-16:10	"Detection of harmful residues in honey using terahertz time-domain spectroscopy"	**Maria Massaouti**
16:10-16:30	"Sensitivity of a new 1,8-naphthalimide cation sensor as function of PET blocking and complex binding constant"	**Stanislava Yordanova**
16:30-16:50	"A polyphenol biosensor realized by laser printing technology"	**Marianneza Chatzipetrou**

Friday October 25th: Biomediators *immobilisation processes for biosensors*

9:00-10:00	"Laser printing and immobilization of biomolecules"	**Ioanna Zergioti (invited)**
10:00-10:30	Marie Curie IAPP action "Laser Digital Micro-Nano fabrication for Organic Electronics and Sensor applications"	**Ioanna Zergioti, D. Karnakis, Ph. Delaporte**
10:30-11:00 Coffee Break		
11:00-12:00	"Efficient immobilization of biomolecules on chemically and topographically modified substrates"	**Aggeliki Tserepi (invited)**

III. Abstracts of lectures

1. "Leaf-like Materials capable of solar energy convention by photosynthesis"

Bao-Lian Su
Laboratory of Inorganic Materials Chemistry, University of Namur, BE

This presentation describes the fabrication, via immobilisation of photosynthetically active entities within silica materials, of photobiochemical leaf-like materials capable of the energy conversion as the principal component of a photobioreactor and a biofuel cell.

The photosynthetic activity shows that the material was able to produce oxygen for over a month. The photochemical material was also able to reduce CO_2 into carbohydrates. A part of these photosynthates were excreted into the aqueous phase contained within the pores of silica. By a simple extraction method, these products could be recovered. The molecules excreted by the material were mainly polysaccharides composed of rhamnose, galactose, glucose, xylose and mannose units. Considering that the quantity of sugars increased as a function of time, this photosynthetic material holds much promise in the development of new, green chemical processes. For instance, atmospheric CO_2 could be strategically exploited via this kind of artificial leaf-like materials, as a source of carbon to produce valuable compounds or biofuels while the active biomass is continuously reused. These results constitute a significant advance towards the final goal, long-lasting semi-artificial photobioreactors and biofuel cells that can advantageously exploit solar radiation to convert polluting carbon dioxide into useful biofuels, sugars or medical metabolites and electricity.

2. "Molecular biotechnologies improving the bioreceptorial properties of Photosystem II"

Giuseppina Rea
Institute of Crystallography, National Research Council of Italy, Rome, IT

Oxygenic photosynthetic organisms use light energy to power electron transfer and charge separation across a charge-impermeable lipid membrane. One of the main working photosynthetic units is the pigment-protein complex Photosystem II (PSII) which hosts, among the others, the D1/D2 reaction center (RC) proteins, the oxygen-evolving complex and antenna core complexes. All the photosynthetic redox active components are located within the D1/D2 heterodimer, which is a site of electron tunneling-mediated charge separation, electron transport chain, and solar energy transduction. PSII is gaining renewed interest due to the possibility to exploit the unique features of its structural and functional constituents for the development of optoelectronic devices, such as biosensors and biochips, for diagnostic and monitoring purposes. Some classes of environmental pollutants can inhibit some photosynthetic functions which can be easily measured by optical or electrochemical transductors. As a consequence, an intriguing opportunity is the development of sensor devices, exploiting native or manipulated PSII complexes or ad hoc synthesized polypeptides mimicking the PSII RC proteins as bio-sensing elements. The integration of molecular biology, biotechnology and computational studies can help to realize novel, more sensitive, specific and stable PSII.

3. "Biosensors based on aptamers detection"

Giorgos Tsekenis
Biomedical Research Foundation Academy of Athens, GR

Aptamers are nucleic acid (DNA, RNA or XNA) or peptide molecules that bind to a specific target analyte with a selectivity and specificity that rivals that of antibodies. They have the ability to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Despite the fact that aptameric sequences exist in nature, in riboswitches, artificial aptamers were first developed in the '90s. Since then, they have been used extensively in a wide variety of research, industrial and clinical applications and most importantly as the biotransducer elements in biosensors. This is due to their numerous advantages over antibodies, such as the lower cost and the greater range of the target analytes. Aptamers would have certainly surpassed antibodies by now, if it was not for the lack of available aptameric sequences since they have to be engineered through repeated rounds of in vitro selection, SELEX (systematic evolution of ligands by exponential enrichment) from a large random sequence pool. The lecture will highlight the advantages of aptamers over antibodies and present a number of selected applications to illustrate their endless possibilities. At the same time, the drawbacks of aptameric sequences will be presented along with their future perspectives.

4. "Introduction and Overview of Biosensor"

Ismail Hakki Boyaci
Hacettepe University, Food Research Center, Ankara, TR

Biosensor is defined as an analytical device incorporating a biological material or a biomimic (e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids etc.), intimately associated with or integrated within a

physicochemical transducer or transducing microsystem, which may be optical, electrochemical, thermometric, piezoelectric or magnetic. Biosensors combine the selectivity of biology with the processing power of modern microelectronics and optoelectronics to offer powerful new analytical tools with major applications in medicine, environmental diagnostics and the food and processing industries. Biosensors are finding use in increasingly broader ranges of application. The following list describes some of the current applications.

• Clinical diagnosis and biomedicine
• Farm, garden and veterinary analysis
• Process control: fermentation control and analysis
• Food and drink production and analysis
• Microbiology: bacterial and viral analysis
• Pharmaceutical and drug analysis
• Industrial effluent control
• Pollution control and monitoring
• Mining, industrial and toxic gases
• Military applications

The rapid development of biosensor for biochemical analysis has been greatly promoted by the progress of microfabrication techniques and microchemical systems using these devices have attracted much attention of scientists and engineers. Recent advantages in nanobiotechnologies (surface functionalisation and patterning, detection, microfluidics, integration) tools (cell manipulation and sensing) will have a decisive impact on the performance of the new generation biosensors and biochips. The new possibilities provided by biosensors (high sensitivity, massively parallel analysis) offer strong opportunities for the implementation of current EU policies strategies and action plan, in particular for those related to the European Environment and Health Strategy, the Life Science and Biotechnology Action Plan and the Environmental Technology Action Plans as well as security. The use of novel sensor techniques, related to measurement and evaluation of exposure to toxic substances, has also a decisive importance in the field of security in general.

5. "Photosynthesis-based biosensors"

Touloupakis Eleftherios
Institute of Ecosystem Study, National Research Council, Florence, IT

Practical monitoring programs require rapid, simple and low-cost screening procedures for the detection of harmful chemicals in aquatic and soil environments. Biosensors are promising biotools, alternative or complementary to conventional analytical techniques, for fast, simple, cheap and reliable screening. Environmental technology is the field where photosystem-based biosensors find most of the applications. Herbicides are toxic chemicals widely used in agriculture, since they provide a low-cost weed control. Photosynthesis inhibition is a reliable indicator that rapidly demonstrates the toxic effect of herbicides. Several photosynthetic materials such as chloroplasts, thylakoids, photosystems or whole cells (cyanobacteria and eukaryotic microalgae) have been employed as bioreceptors for the development of electrochemical and optical biosensors. The main advantages of the use of the photosynthetic material is the high sensitivity, the short duration of the test and the availability.

6. "Monolithic silicon interferometric optoelectronic platform for label-free multi-analyte biosensing applications"

Ioannis Raptis
Department of Microelectronics, NCSR 'Demokritos', Athens, GR

Miniaturized bioanalytical devices find wide applications ranging from blood tests to food safety and environmental monitoring. Such devices in the form of hand held personal laboratories can provide point-of-need monitoring through their miniaturization, multi-analyte detection and sensitivity capabilities. Optical detection in biosensors is superior in many respects to other types of sensing based on alternative signal transduction techniques, especially when both sensitivity and label free detection is sought. The main drawback of optical biosensing transducers relates to the unresolved manufacturability issues encountered when attempting monolithic integration of the light source. If the mature silicon processing technology could be used to monolithically integrate optical components, including light emitting devices, into complete photonic sensors, then the lab on a chip concept would materialize into a robust and affordable way. Here, we describe and demonstrate a bioanalytical device consisting of a monolithic silicon optocoupler appropriately engineered as a planar interferometric microchip. The optical microchip monolithically integrates silicon light emitting diodes and detectors optically coupled through silicon nitride waveguides designed to form Mach-Zehnder interferometers. Multi-analyte label free detection of model assays and of analytes with high practical use are demonstrated.

7. "Characterising biosensors and biosolar cells as photovoltaic devices"

Raoul Frese
Department of Physics and Astronomy, VU University Amsterdam, NL

Biosensors are biohybrid devices consisting of biological preparations interconnected with non-biological materials. Within the PHOTOTECH framework we research the application of photosynthetic complexes and membranes within biohybrid devices for photovoltaics and sensing pollutants. In both cases, the operation of the device depends on the photoconversion of excited states

into charge transfers by electron tunneling, currents and charge mediation. These processes are not just similar to solar cells, they are exactly the same and therefore the primary performance of a biosensor should be evaluated as a solar cell. On top of this, depending on the sensing functionality, the secondary performance depends on the sensitivity of the photovoltaic response for the compounds to be detected. Primarily, biosensors can be characterized most easily by means of electrochemistry to inform on the interconnection of biological material with a conducting electrode. Issues to address are the internal quantum yield, stability, sensitivity and homogeneity of preparation. Secondary, the material can be incorporated in a solar cell to obtain the external quantum yield, fill factor and electrical power generated. In this lecture I will address several ways of investigating these properties including general biophysical techniques such as atomic force microscopy and single molecule spectroscopy.

8. "Electron transfer in biophotoelectrochemical devices"

Nicolas Plumeré
Ruhr-Universität Bochum, Center for Electrochemical Sciences, DE

Photosynthetic proteins are primarily implemented in biophotoelectrochemical devices for biosensing and energy conversion applications. The performances of these devices rely on the electronic communication between an electrode and the photosynthetic proteins. This communication may be achieved either by direct or mediated electron transfer. In the latter case electron relays are applied for (i) protein and mediator freely diffusing in solution, (ii) proteins bound to electrode surface or (iii) both protein and mediator surface confined. Approaches for the quantitative analysis of the electron transfer rates between redox enzyme and electron relays as well as for the direct electron transfer situation will be presented. Investigation of possible charge recombination processes will be discussed as well.
The key properties, beside fast electron transfer, of the ideal mediator for a given sensing concept will be discussed. For efficient energy conversion, the electron transfer should take place with maximal current density at low overpotential. To illustrate the desired parameters of an electron relay, the example of a biophotovoltaic cell based on photosynthetic protein complexes will be given. In technological applications, the electron relay, beyond its role in electron transfer, may also be used for shielding the biocatalysts from various deactivation pathways.

9. "Laser printing and immobilization of biomolecules"

Ioanna Zergioti
Physics Department, National Technical University of Athens, GR

In this work Laser Induced Forward Transfer (LIFT) is utilised for both the printing and the immobilization of biomaterials on sensor substrates. LIFT is a direct write technique, which enables the direct immobilization of biomaterials, on rough substrates, without any functionalization layer. Side view time-resolved imaging of the transfer process was conducted by pump-probe setup, using a ns Nd:YAG laser at 266 nm as a pump and a ns Nd:YAG laser at 532 nm as a probe light source. The measured velocities were from 30 m/s to 200 m/s of the liquid jets which corresponds to an impact pressure ranging for 0.45 to 35 MPa, i.e., almost 30 times higher than the pressures attained with other conventional printing methods (e.g., the maximum impact pressure for conventional ink jet printing is about 1MPa). The optimum laser printing energy fluence, for the direct immobilization is defined at 500 mJ/cm^2, corresponding to the an impact pressure is estimated to be 1.2 MPa, which could be considered as the minimum pressure required for forcing the biomaterial within the roughness of the substrate. The LIFT technique was applied for the immobilization of biomolecules for the development of biosensors. Such an example is the laccase enzyme, on Screen Printed Electrodes for the fabrication of an enzymatic biosensor. The SPEs consisted of a counter, a reference and a graphite working electrode, onto which the enzyme was immobilized. The biosensor was characterized towards a polyphenol compound, catechol, as its detection is very important for the food industry.

10. "Laser Digital Micro-Nano fabrication for Organic Electronics and Sensor applications"

I. Zergioti[1], D. Karnakis[2], Ph. Delaporte[3]
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[3] Laboratoire Lasers, Plasmas et Procédés Photoniques, 13288 Marseille, France

LaserMicroFab is a joint Marie Curie Industry Academia Partnership research project, recently launched, and it is based on the knowledge and expertise of two academic partners (National Technical University of Athens (NTUA) and CNRS-LP3) and one SME, Oxford Lasers (OL) through inter-sectorial exchange of knowledge, networking activities and training in the areas of advanced laser processing for organic electronic devices and biosensors. The goal for this project is to develop Laser digital micro-fabrication processes such as selective laser micro and nanopatterning, laser micro-curing and laser micro-printing for precision patterning of complex materials, such as metallic nanoparticle (NP) inks and organic materials. The developed laser processes will be employed for the micro-curing of metallic nanoparticle (NP) interconnects to achieve submicron spatial resolution, for the nanostructuring of ultrathin (<50 nm) layers and for the printing of organic semiconductors for electronics and/or photovoltaics applications. Moreover, patterns of biomolecules will be printed using the laser micro-printing process without compromising the viability of these delicate structures. The success of this project will have a great impact on the market potential of Oxford Lasers' products and the research excellence of NTUA and CNRS-LP3 in the fields of materials engineering, biotechnology and chemical engineering, ensuring its multidisciplinary character. At the end of this project, a full set of

parameters will be established and optimised as an innovative tool for material processing and will be further exploited for new applications and market areas.

11. "Efficient immobilization of biomolecules on chemically and topographically modified substrates"

Aggeliki Tserepi
Institute of Microelectronics, NCSR Demokritos, Athens, GR

Immobilization of biomolecules on substrates has recently attracted considerable attention as an enabling technology for applications ranging from biosensors and BioMEMS to tissue engineering. A method for efficient immobilization of biomolecules on solid supports will be presented based on plasma processing of the substrates. Plasma treatment of surfaces is known to lead to chemical and topographical modification of exposed surfaces. We will show that, on one hand, the plasma-induced chemical modification of a variety of substrates (silicon, glass, polymers) leads to rapid and yet stable immobilization of biomolecules on these substrates, while on the other hand, the high surface area of plasma nanotextured substrates leads to extremely homogeneous deposition of biomolecules, at concentrations much higher than those on flat substrates. Furthermore, in the case that patterned substrates are used, proper plasma modification results, in addition, in selective immobilization of biomolecules on desired areas, achieving biomolecule patterning at densities many orders of magnitude higher than those made possible by commercial spotting systems. Application of this method in the fabrication of protein or DNA microarrays will be presented and the advantages of its implementation in various analytical microdevices will be discussed.

IV. Abstracts of oral presentations

1. "Bio-photovoltaics based on hybrid systems of reaction centers and diamond"

Roberta Caterino, *Réka Csiki, Matthias Sachsenhauser, Martin Stutzmann, Anna Cattani-Scholz, Jose A. Garrido*
Walter Schottky Institut, Technische Universität München, Germany

Photosynthetic reaction centers (RCs) are protein complexes responsible for solar energy harvesting in plants, bacteria, and algae. The high efficiency of these proteins in achieving charge separation under photo-stimulation has attracted interest in using RCs as a functional unit in bio-solar cells. However, the complexity of the charge transfer between the biological species and the inorganic electrode typically leads to low values of the measured photocurrents in such systems. A great effort has been done in the last years to optimize the immobilization of RCs on several surfaces making use of suitable linker molecules. Recently, we have suggested that diamond is an interesting alternative to metal electrodes in these bio-hybrid devices, as it exhibits excellent electrochemical properties and, at the same time, it provides a suitable surface for covalent immobilization. In this contribution, we will discuss the immobilization of RCs from purple bacteria on highly B-doped nanocrystalline and polycrystalline diamond electrodes using various grafting protocols. We have studied the photocurrent signal generated from the photo-excitation of immobilized RCs in the presence of cytochrome C and coenzyme Q0 in solution. We have found that the role of these latter two species in the charge transfer is similar to the role they play in the natural environment of RCs, with cytochrome C shuttling the low-energy electrons from the electrode to the RCs P-side and Q0 extracting the high energy electron from the Q-side of the RCs and shuttling it into the electrolytic solution. A deeper insight into these processes is provided by studying the photocurrent signal as a function of the concentration of these two mediators in solution, in order to investigate the processes taking place at the interface between RCs and electrodes as well as within the species in the electrolyte. We have also investigated the dependence of the photocurrent signal on the voltage applied between reference and working electrode, enabling a deeper understanding of the different steps involved in the charge transfer induced by photo-excitation and how the energetic level of electrodes and redox species can be tuned to maximize the measured photocurrent levels. This work demonstrates recent progress in the use of diamond electrodes in bio-hybrid systems for solar energy harvesting.

2. "Construction of photovoltaic cells based on Rhodobacter sphaeroides reaction centers"

Rafal Bialek, *Krzysztof Gibasiewicz*
Faculty of Physics, Adam Mickiewicz University, Poznan, PO

It has been widely used to study energy and electron transfer in photosynthetic reaction centers (RCs) of purple bacterium Rhodobacter sphaeroides. First steps in photosynthetic reaction center are absorption of photon and electron transfer from dimeric bacteriochlorophyll P to the nearby electron acceptor, bacteriopheophytin HA, forming the charge-separated state P+HA-. In RCs prepared in a specific way, electron from HA- comes back to P+, forming the ground or excited singlet state of both molecules or triplet state 3P. There is some information suggesting that energy from triplet state could be used to produce electricity by positioning RCs on the titanium dioxide layer. There is a variety of purple bacteria mutants described in literature. They are characterized by different quantum yield of triplet state formation and probably different efficiency in solar cells. During the presentation some preliminary results of the research carried out using nanosecond transient absorption spectroscopy and stationary absorption spectroscopy will be presented. The used research methods will be also discussed.

3. "Screening of electricity producing profile of various photosynthetic microorganisms"

Bilge Hilal Cadirci
Faculty of Engineering and Natural Sciences, Department of Bioengineering, Gaziosmanpasa University, TR

Bioengineering mimics "life" to facilitate life for human being. Life is a kind of results of energy pathways. Energy never dissapears, it is just converted into different forms. A fuel cell is a device that converts the chemical energy from a fuel into electricity through a chemical reaction with oxygen or another oxidizing agent. In microbial fuel cell the biochemical energy from microbial biomass is the source of fuel. If the microorganisms is photosynthetic, the energy originally comes from sunlight and then its called photosynthetic microbial fuel cell (PMFC). In this work we aimed to screen electicity production potentials of various photosynthetic microorganisms.

4. "Photocurrent generated by photosynthetic reaction center/carbon nanotube/ito bio-nanocomposite"

Tibor Szabó, László Nagy
Department of Medical Physics and Informatics, University of Szeged, HU

Intensive studies have sown recently that photosynthetic proteins purified from plants (PS-I and PS-II) and from purple bacteria bind successfully to nanostructures while their functional activity is largely retained. Current researches are focussing on finding the best bio-nanocomposite sample preparations and experimental conditions for efficient energy conversion and for the stability of the systems. In our studies reaction center proteins (RC) purified from purple bacterium Rhodobacter sphaeroides were bound successfully to amine- and carboxy-functionalized multiwalled carbon nanotubes (MWNTs) immobilized onto the surface of ITO by using specific silane. Structural (SEM, AFM) and functional (flash induced absorption change and conductivity) techniques have shown that RCs can be bound effectively to the functionalized carbon nanotubes (CNT). An electrochemical cell with three electrodes (reference Ag+/AgCl, counter platinum and the working sample) was designed especially for measuring the photocurrent generated by this composite material. Several hundreds of nA photocurrent was measured with fully active RCs while the current was missing when the RC turnover was disrupted by depleting the electron acceptor quinones.

5. "A new thiol-coated interface for the development of an aptasensor for lysozyme"

Iuliana Mihai, Alis Vezeanu, Alina Vasilescu
International Centre of Biodynamics, Bucharest, Romania

The conditions used for manufacturing a platform for biosensing applications need to be carefully chosen because they can affect the efficiency of the resulting sensor. A new aptasensor based on Surface Plasmon Resonance (SPR) has been recently developed by our team for the detection of lysozyme. The sensor relied on the use of gold interfaces coated with a long, carboxyl-ended thiol containing 6 ethylene glycol groups, very useful for minimizing non-specific binding. Here we report the development and characterization of a new thiol coating allowing controlled and efficient immobilization of biorecognition elements and minimum non-specific adsorption. The coating is obtained from a classic mixture of a carboxyl: hydroxyl ended thiols in a 1:20 ratio. In this study, the previously used carboxyl-ended thiol was mixed with a much shorter compound, HS-(CH2)2EG-OH that was recently synthetized and characterized. The non-fouling properties of mixed thiol layers evaluated with respect to lysozyme were found to be greatly influenced by the cleaning procedure of the gold SPR chips. We functionalized further the interfaces with Neutravidin, as a way to obtain versatile affinity platforms for biosensors. The immobilization capacity of the sensor coated with the mixed SAM decreases only by 18% compared with the homogeneous carboxyl-ended SAM. Studies with several proteins with different molecular weights and isoelectric points demonstrated the efficiency of the mixed SAM in removing the nonspecific adsorption, as well as a good operational stability upon several testing/regeneration cycles. Proof-of-concept experiments were performed using thiol-modified interfaces for the development of an aptamer-based biosensor for lysozyme that is amenable to both SPR and electrochemical investigations (e.g by faradaic electrochemical impedance spectroscopy).

6. "Challenges in the development of an electrochemical (bio)sensor for allergen proteins detection"

Alis Vezeanu, Iuliana Mihai, Alina Vasilescu
International Centre of Biodynamics, Bucharest, Romania

Food allergy is an immune-based disease that has become a serious problem and is defined as an adverse reaction that involves IgE antibodies to one or more allergen proteins. Therefore, due to their importance, the detection of these allergen proteins has lead to the development of a variety of analytical methods based on chromatographic, spectroscopic or biological techniques. Most of these methods are time consuming and have low sensitivity. This work presents the challenges in the development of electrochemical biosensors for the sensitive detection of allergens such as gliadin and lysozyme. Specificity was ensured by the use of an antibody for gliadin and an aptamer for lysozyme, respectively. To monitor the sensor building steps and for the detection of allergenswe performed cyclic voltammetry (CV) and impedance measurements (EIS) in the presence of ferri/ferrocyanide redox couple. We have investigated two types of electrode materials: gold electrodes covered by a Self Assembled Monolayer formed from a mixture of a hydroxyl-ended and a carboxyl-ended thiol (a) and screen-printed carbon electrodes modified with the diazonium salt of p-aminobenzoic acid (b). Deposition of diazonium salts by electrochemical reduction offers a fast and easy way to form uniform, high stability layers with a wide range of functional groups. We took

advantage of the carboxyl groups grafted on the surface of both materials to immobilize the bio-recognition elements-gliadin or a lysozyme aptamer. Non-specific adsorption was a serious problem. To minimize it, we coated the gold sensor with a thiol containing ethylene glycol or we adsorbed Bovine Serum Albumin. Coating the electrode with a thiol that contains ethylene glycol groups diminished the non-specific adsorption of lysozyme by 91.2% as determined by EIS. In a similar approach we have considered in the case of screen-printed carbon electrodes Bovine Serum Albumin (BSA) and triethylene glycol monoamine. The best strategy to maximize the specific/non specific signal ratio will be highlighted.

7. "Full automation of a rapid screening test for early warning measurement of phytotoxicity in water samples based on photosynthetic algae"

Annalisa Tortelli, Sergio Bodini
Systea SpA, Anagni(FR), Italy

The control and monitoring of herbicide pollution of water and groundwater bodies is a key issue for the protection of human health and safety. Long term phytotoxic effects of environmental samples are commonly assessed by fresh water algal growth inhibition assays (ISO 8692). However, in recent years, the necessity of reducing measurement times in order to rapidly detect changes in water quality and allow timely intervention, motivated the improvement of rapid screening tests based on photosynthetic biomediators, able to detect the presence of herbicide activity in shorter time, via the kinetic analysis of Chlorophyll a fluorescence induction curves (Stirbet and Govindjee, 2011). In particular, the unicellular green alga Chlamydomonas reinhardtii was recognized as an effective monitoring organism for herbicide detection in water and wastewater samples (Scognamiglio et al., 2009). This is accomplished by combining specified volumes of the test sample with the freshwater algal suspension in a test tube and measuring the variation of the quantum yield of primary PSII photochemistry. On the other hand, being based on discontinuous sampling strategies, manual procedures provide only temporary control of tested waters, whereas on line monitoring, via completely automated systems, allows to increase the analytical throughput and obtain rapid, accurate and reproducible results. Aiming at this, an original direct reading multiparametric analyzer (Easychem TOX on-line, Systea SpA) was adapted to autonomously perform C. reinhardtii bioassay and thus tested for fully automated phytotoxicity measurements of water and wastewater samples. The analyzer is a random-access platform equipped with colour touchscreen LCD and housed in an industrial cabinet, comprising two refrigerated compartments for reagents, calibrants and controls, an illuminated compartment for microalgae, a mechanical arm for aspiration, transferring and dispensing of reagents and samples and a thermostated reaction plate holding 80 positions, incorporated with a fluorimeter and integrated with an automated cuvette washing station. Wild type strains of C. reinhardtii were grown and stabilized offline using a well tested protocol. In the automated algal toxicity assay, C. reinhardtii were then exposed to water samples in the dark for 15 minutes and the effect of sample toxicity was evaluated by comparing Chlorophyll a fluorescence induction transients of samples with those emitted by blanks, performed on reference water. The detection system is able to collect 2,000 fluorescence emission measurements between 0.1 msec and 10 sec after excitation, demonstrating the effect of the presence of herbicides on fluorescence emission. Fluorescence kinetic curves of blanks and samples are automatically processed by the software according to an ad-hoc algorithm and toxicity is calculated as inhibition percentage of the relative fluorescence variation. Within the analyzer, the microorganisms preserved, for a week, a measurable fluorescence signal and an unaltered sensitivity to different types of reference compounds, such as diuron, linuron and atrazine.

8. "Detection of harmful residues in honey using terahertz time-domain spectroscopy"

M. Massaouti[a,*], C. Daskalaki[a], and S. Tzortzakis[a, b]
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In this work, terahertz time-domain spectroscopy (THz-TDS) has been applied to detect harmful chemical residues in pure honey. Three antibiotics, commonly used in bee industry -sulfapyridine, sulfathiazole and tetracycline- and two different acaricides -coumaphos and amitraz- were studied and characterized in the THz frequency regime from 0.4 up to 6.0 THz. All chemical substances present distinct absorption peaks, showing the ability of THz spectroscopy to discriminate different substances strictly related to food safety issues. In addition, THz transmission measurements through mixtures of pure honey with antibiotics have been performed. The results showed that antibiotic residues are traceable in honey at relatively low concentrations thanks to their distinct THz fingerprints. Finally, multiple antibiotics were identified in their mixture with pure honey, pointing out the potential of the technique to be used in the near future as a fast real-time technique for detecting multi-residues in food industry.

9. "Sensitivity of a new 1,8-naphthalimide cation sensor as function of PET blocking and complex binding constant"

Stanislava Yordanova[1], Stanimir Stoyanov[1], Stanislav Stanimirov[1], Ivo Grabchev[2], Ivan Petkov[1]
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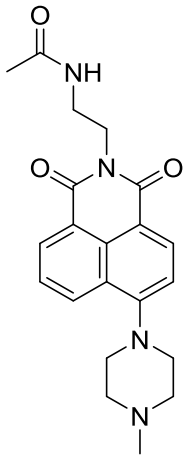
Naphthalimide derivatives are a special class of environmentally sensitive fluorophores. Because of their strong yellow-green fluorescence and good photostability 4-amino-1,8-naphthalimide derivatives have found application in a number of areas

including fluorescent bio-markers, fluorescence sensors and switchers and many more. Moreover, these properties are essential when employing such devices in real-time and on-line analyses. 1,8-naphthalimide is one of the best reported fluorescent sensors due to good photostability, strong fluorescence, large Stokes Shifts and easy modification.



Here we present the functional properties of new 4-amino-1,8-naphthalimide compound. Its functional capacities as highly sensitive PET sensor for different metal cations and protons have been outlined.

10. "A polyphenol biosensor realized by laser printing technology"

Marianneza Chatzipetrou, E. Touloupakis, I. Zergioti
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An amperometric biosensor sensitive towards phenolic compounds, using the enzymes as biorecognition elements, was developed. The enzymes were successfully immobilized in active form onto non functionalized screen printed electrodes by using the laser direct immobilization technology. This type of immobilization established efficient electrochemical contact between the enzymes and the electrodes surface. The immobilized enzymes were characterized towards phenolic compounds in solution. A typical phenolic compound, is catechol, at which the biosensor's sensitivity was found to be 0.43 nA ± 0.04 nA with Laccase as biorecognition element. This biosensor permits the detection of polyphenols in aqueous solutions at concentrations in the nanomolar range.

V. Abstracts of posters

1. "Optical Bioprobe for Detection of Veterinary Antibiotic Residues in Milk Samples"

Gerardo Grasso
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The challenge of food safety requires the employment of field analytical tools based on rapid and effective methods for early identification of chemical-toxicological hazards. Biosensoristic devices possess suitable characteristics in terms of analytical performance, easy use, capacity of providing real-time results, minimised use of reagents and cost-effectiveness. In particular, my current research activity focuses on whole cell bioprobes and the design and development of a optical fluorescent bioprobe based on transgenic microbial cells for the detection of fluoroquinolones in milk samples. The uncontrolled use of veterinary antibiotics in dairy animals poses the problem of residues in milk: when residues exceed the maximum residue limits (MRLs), milk is unfit for consumption. Therefore the progresses in genetic engineering allow the modification of unicellular microorganisms into inducible bioreporters usable as efficient biomediators for semi or quantitative optical analyses (*e.g.* fluoroquinolones) through a biosensoristic device. Using a specific DNA construct (inducible promoter fused to a reporter gene *e.g.* GFP gene), genetically engineered *E. coli* B strain ATCC 11303 cells will be coupled with a suitable transduction element. Parameters likely to influence the analytical detection will be tested and optimized as well as the proper type of immobilization (if necessary). The possibility of designing disposable or reusable biomediators will be also considered.

2. "Optimization of method's parameters for detection of residues of pesticides in bovine milk"

Gloria Rossi
CNR, Rome, IT

The use of pesticides in agricultural productions is still an important issue needing risk management to minimize environmental and human health risks. The regular use of pesticides, e.g. herbicides, may determine the accumulation of (mixtures of) residues in the environment (e.g. water, soil); such residues, in their turn, may contaminate food chains during primary production. Photosynthetic organisms are excellent biomediators for the sensoristic detection of herbicides because most of the herbicides interfere with the photosynthesis process by acting directly with a protein of photosystem II (PSII) and blocking the electron transport chain. Literature data report results on the method's application to standard Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) solutions [1-2]. My current activity is the optimization of method's parameters of the optical biosensor based on the photosynthesis process of a unicellular alga (Chlamydomonas Reinhardtii).
This research is carried out in the context of technological platform of ALERT -Integrated System of biosensors and sensors (BEST) for monitoring the health, the quality and traceability of bovine milk- (www.alert2015.it). The aim of this activity is the application of the probe in complex real matrices like milk and the development of a method for residues of interest for the dairy chain.

3. "From whole cells towards photosynthetic reaction centers: 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.

4. "Use of microalgae and enzymes for the development of a multiarray biosensor based on fluorescence"

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A biosensor is a device which use in biological recognition element (bioreceptor) retained in direct spatial contact with a transduction system. In this work were used microalgae and enzymes, such as urease, tyrosinase, acetylcholinesterase and b-galactosidase, to develop a multiarray biosensor based on fluorescence, able to determine the presence in milk of pollutants and compounds that determine the quality. Experiments were performed in buffer to check LODs and subsequently, each experiment was replicated in milk. Excellent results were obtained with microalgae and urease, while with the other enzymes trials are still in progress.

5."Organophoshorus pesticide detection by cholinesterase biosensors based on SPE"

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[b] *Consorzio Interuniversitario Biostrutture e Biosistemi "INBB", Rome, Italy;*
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Organophosphorus pesticides are largely used due to their high insecticidal activity and relatively low persistence. Their toxicity is due to the inhibitory effect on acetylcholinesterase, a key enzyme for the nerve transmission. The detection of organophosphorus insecticides is generally carried out using gas or liquid chromatography, that requires skilled personnel, laboratory set-up and expensive instrumentation . An alternative analytical system is the use of biosensors, which are cost effective, miniaturized and friendly to use.
In the present work, a biosensor for organophosphate detection, based on *butyrylcholinesterase* enzyme inhibition was developed. By this device, the amount of the organophosphate present in the sample is quantified measuring the enzymatic activity before and after the biosensor exposure to the sample. The enzyme was immobilized by cross-linking glutaraldehyde,

Nafion and bovine serum albumin on screen-printed electrodes modified with the electrochemical mediator Prussian Blue (PB-SPE). The use of Prussian Blue allows us to detect the enzymatic activity at low applied potential (+200 mV vs Ag/AgCl). The enzymatic membrane was optimised and the biosensor was challenged in amperometric mode toward paraoxon, reaching a detection limit of 0.14 ppb (10% of inhibition) and linear range up to 5 ppb.
The biosensor was then re-optimised in order to be assembled in a flow system, for an easy automatisation. For this purpose, the biosensor was inserted in a thin-layer cell and the effect of the flow rate during the substrate measurement, and the incubation time (the time of the reaction between enzyme and inhibitor) were optimised to 0.12 ml/min and 0.25 ml/min, respectively. The system can detect paraoxon with a detection limit of 1 ppb (10% of inhibition) and linear range up to 10 ppb. The developed system was also applied for monitoring tap, river and lake water with satisfactory results in terms of accuracy.