Divisione di Chimica Analitica
Società Chimica Italiana

Gruppo Divisionale Sensori

III Workshop
Università degli Studi di Firenze
26 – 28 Ottobre 2010

BOOK OF ABSTRACTS

GS2010

Giornate di Studio dedicate al Prof. Marco Mascini
in occasione dei suoi 70 anni

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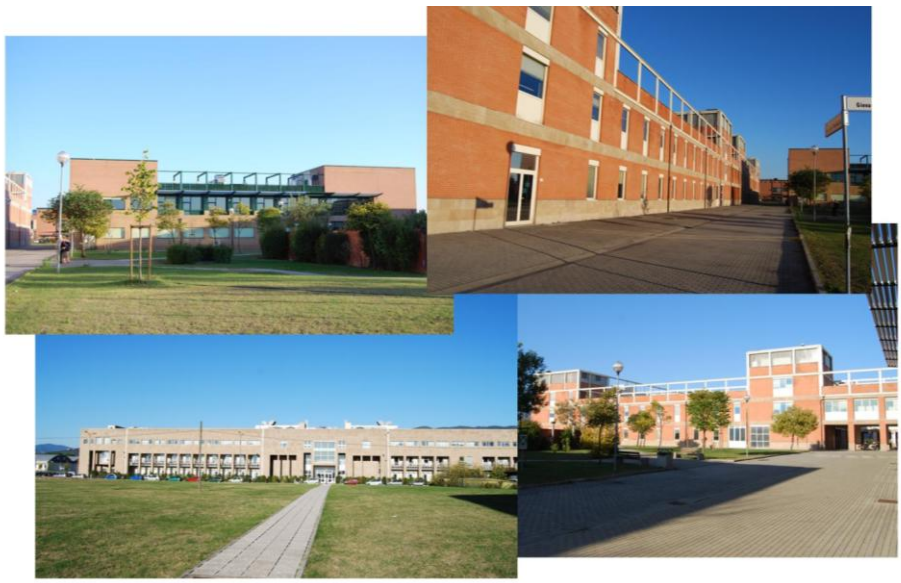

AULA MAGNA DEL PLESSO DIDATTICO

POLO SCIENTIFICO E TECNOLOGICO DI SESTO FIORENTINO

Via Gilberto Bernardini, 6- 50019 Sesto Fiorentino (Fi)

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INVITED

SPEAKERS

BIOSENSORS RESEARCH IN FIRENZE IN THE LAST 25 YEARS

Marco Mascini

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This lecture will be dedicated to a survey of the major results and activities realised in the last 25 years in our Group in Firenze.
Then starting from the preparation of screen printing electrodes we faced several important analytical problems solved by exploiting the use of bioanalytical elements immobilized over several kind of transducers, screen printed electrodes, but also optical devices and piezoelectrical quartz nanobalances, then realizing and applying the Biosensors technology.
Three major applications Area of Biosensors have been persecuted. 1)The Diagnostic area in Medicine, in this case starting from the artificial pancreas problem (glucose sensor) then the interesting goal to succeed to personalise the dialysis of blood with sensors for urea, uric acid etc. then now recently again by exploiting the aptamers realising sensors for a rapid detection of Activated C Protein, Neopterin, Thyroid-stimulating hormone (TSH) etc.
2) the area of Environment where several PhD students get their degree just exploiting the possibility to detect pesticides of a specific enzyme the Acetylcholinesterase or more recently the evaluation of DNA Damage exploiting DNA from calf thimus and the electrochemical signal of the guanine oxidation on graphite just through the screen printing facility.
3) The area of Food, in this case we considered the quality of food (freshness sensors and/or the presence of genetically modified food) , and the detection of food safety like in this case the detection of low concentration of bacteria (Listeria, Salmonella etc.).
Finally the recent advances in the field of molecular recognition with the introduction of Aptamers , now divided in two distinct classes, Oligonucleotides Aptamers and Peptide Aptamers from which we are waiting for new devices more stable, more sensitive more selective !
Then the match is over and we can continue to look for!

BIOSENSORS: MEETING THE DEMAND FOR PERSONAL DIAGNOSTICS

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The world market for biosensors, in 2009, was just under US$13 billion, with nearly nine tenths of that still accounted for by glucose measurement. This extraordinary dominance by a single biosensor type is driven by the exceptional needs of people with diabetes combined with the success of biosensors in meeting their demands for an appropriate product. The overall *in vitro* diagnostics market is currently considered to be worth around $40 billion, while recent estimates suggest that the theranostics (companion diagnostics) market is potentially worth a staggering $72 billion. Diabetes is the fastest growing chronic disease in the World, with Asia now home to four of the five largest diabetic populations and 2% of the World's population afflicted. Heart disease and stroke kill around 17 million people each year, accounting for one-third of all deaths globally. By 2020, they will be the leading cause of both death and disability worldwide, with fatalities projected to increase to greater than 24 million by 2030. Infectious diseases account for over 16% of worldwide deaths, comprising those associated with poverty such as malaria, HIV/AIDS and TB (1/3rd of world population infected and 2 million deaths) and epidemic & emerging diseases such as meningitis, cholera, yellow fever, flu and antibiotic resistant infections. Around 12% of deaths worldwide are from cancer (Africa 4%, North America 23%; UK 24%) while approximately 11 million people worldwide are diagnosed each year with cancer (45% are in Asia). Finally, society needs to make adequate provision for the proper care of an aging population, the *demographic time bomb*, with the proportion of people in the world aged ≥60 yrs old expected to rise from the current 10%, to 22% by 2050. Current global healthcare spending is more than $5 trillion per year and is is growing fast (15% of GDP for USA, 8% of GDP for Europe). These combined needs of the individual with societal and economic pressures will force a paradigm change in the way disease is managed, with the future focus on prevention, diagnosis and less expensive, decentralised technologies; in many cases these will be a matter of individual consumer choice. Home testing has been revolutionised by the introduction of colorimetric test strips, electrochemical biosensors, lateral-flow immunoassays and the recent commercialisation of self-implantable glucose biosensors. The completion of the Human Genome project has enable rapid advances in molecular diagnostics and pharmacogenetics, unraveling new pathways and revealing novel potential biomarkers. While personalised medicine is still in its infancy, the poor efficacy of many current pharmaceuticals is a strong driver for combining diagnostics with therapy. Molecular diagnostics to enable better administration of anticoagulant therapy, predict drug metabolism and detect mutations that identify patients likely to respond to cancer therapy, have already met with notable success. The combination of diagnostics with pharmaceuticals not only furnishes immense clinical benefit, but provides a new financial model to drive forward the development of new sensors. With over 6,000 papers published on biosensors in 2009, a plethora of patents and a myriad of new product development announcements, we see visions of the future including exploitation of nanotechnology, synthetic biology and the creation of plastic bioelectronics. This presentation will seek review this emerging new paradigm in biosensor technology.

METAL OXIDE NANOWIRES FOR CHEMICAL AND BIO-SENSING

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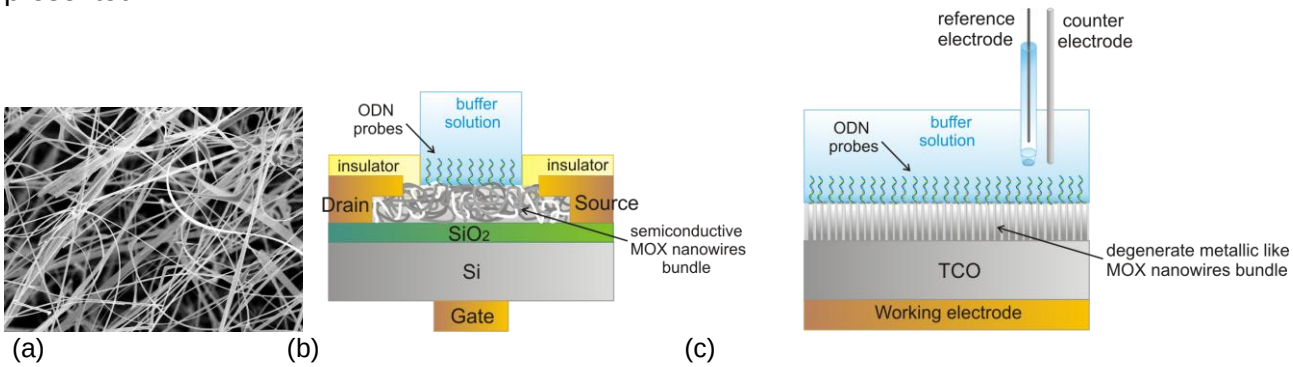
Quasi one-dimensional (1D) semiconductor nanocrystals represent the forefront of today's solid state physics and technology. Top-down methods to fabricate 1D nano-scale structures do not guarantee a sufficiently low dimensionality, the resolution limit of nano-lithographic techniques being around 100 nm. Bottom-up techniques have instead great potentials in terms of crystalline perfection, cost and high productivity. One of the most promising self-assembly method is the so-called evaporation-condensation vapour-solid (VS) and metal catalyst assisted vapour-liquid-solid (VLS) mechanism, proposed by Wagner and Ellis in 1964 for silicon whisker growth [1]. Following these routes quasi 1D nanostructures (so called nanobelts or nanoribbons) of semiconducting oxides of Zn, Sn, In, Cd and Ga have been successfully synthesized first by Pan et al. [2]. Shortly afterwards they have become the focus of intensive investigation as potential building blocks for nanoscale devices and sensors. In these studies the metal oxide NWs are typically not intentionally doped, and the carriers are normally generated by structural defects such as oxygen deficiencies. As a result, the devices behave as '1-D' wide band gap semiconductors whose current flow is extremely sensitive to minor surface perturbations. When employed as a chemical gas sensor the performances are influenced by the surrounding environment [3], when employed as biosensor after surface grafting with specific receptors, any biological macromolecules bound to the surface and undergoing a binding event with conformational change or change of a charge state, may perturb the current flow in the nanowires –fig. 1(b)-

On the other hand, intentional doping can provide semi-metallic behavior, greatly affecting the device properties and yielding new device applications, as electrodes in organic solar cells, field emission sources and thin film transistors. Indeed in deep characterization and functional applications of these innovative electrodes are still in their infancy, a field in which they could represent a breakthrough is electrochemical biosensing in the configuration showed in fig. 1(c).

State of the art and recent achievements obtained by using highly reactive metal oxides will be presented.



(a) (b) (c)

Figure 1. SEM image of 1D nws (a), working principles of semiconducting nanowires based electrical biosensors (b) and of an electrochemical biosensor incorporating semimetallic nanowires electrodes (c)

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ELECTROCHEMICAL BIOSENSING WITH NANOPARTICLES

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The need for nucleic acid and protein based diagnostic tests has increased enormously in the last few years and the design of nanoparticles with special optical and electrochemical properties is bringing significant advantages in several fields being diagnostic one of the most important. In this context biosensor technology represents an interesting alternative for the development of efficient, fast, low-cost and user-friendly diagnostic devices. Between different biosensing alternatives the nanotechnology and nanomaterial oriented biosensors represent a very attractive tool for clinical applications.

DNA, protein and even cell detections methodologies with interest for diagnostics and based on nonoparticles will be described. The developed devices are based on the use of special platforms which allows their future applications and extension in diagnostic. In addition the nanoparticle based biosensors are being offered as excellent screening alternatives to sophisticate and high cost equipments that require well prepared professionals for their use, including data treatment, prior obtaining of final results with interest for diagnostic and treatment.

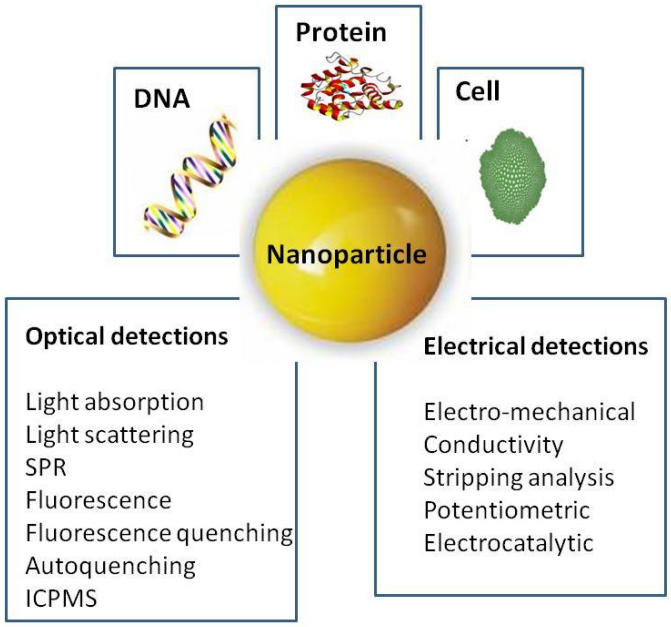

Figure 1. Schematic (not in scale) of some of the optical and electrical detection alternatives that are being used for DNA, proteins and cells analysis thanks to the use of nanoparticles. SPR: surface plasmon resonance; ICP-MS: Inductively coupled plasma mass spectroscopy. [1] .

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ORALS

BIO-CHEMILUMINESCENT MINIATURIZED BIOSENSORS

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The Point-Of-Care (POC) approach is based on portable devices suitable to perform the analysis directly where it is required or where the sample is obtained. POC, which can provide significant advantages in the clinical field for its ability to provide accurate diagnosis at the patient bedside, can find applications in other fields, such as critical medicine, bio-terrorism, developing countries, food and environmental analyses, space station and telemedicine.
A POC device should combine portability, minimum sample pre-treatment and ability to perform highly sensitive multiplexed assays in a short time. Microfluidic integrated systems relying on biospecific recognition reactions (e.g., immunological reactions, nucleic acids hybridization) and ultrasensitive bio-chemiluminescence (BL-CL) detection techniques represent one of the most promising options[1]. Although several miniaturized analytical systems were developed, in most cases the miniaturization of the analytical format was not paralleled by an adequate miniaturization of the overall analytical device (e.g., use of laboratory instrumentation for signal detection).
This work describes the development of POC devices exploiting "contact" BL-CL detection, in which the analytical signal is produced directly in the surface of an imaging light sensor able to localize and quantify the signal. In particular, the analytes are captured and detected in different positions of a transparent solid support, which is in contact, through a tapered fiber optic faceplate, with a portable cooled highly sensitive CCD sensor. "Contact" imaging, which provides high light collection efficiency, has been exploited in different analytical devices[2].
A microfluidics-based device was developed for the diagnosis of parvovirus B19 (B19V) infection. In particular, target B19V DNA is captured by a specific peptide nucleic acid (PNA) probe immobilized on a glass surface, and then revealed by exploiting an enzyme label suitable for CL detection. The limit of detection (50 fmol/mL of B19V DNA) is comparable with that obtained with standard laboratory methodologies, with an overall assay time of 30 min.
A new portable biodevice containing genetically engineered BL whole-cell biosensors[3] was developed for multiplexed detection of environmental pollutants (e.g., compounds with hormone-like activity, heavy metals…). The cells were modified with the introduction of the gene encoding for a BL reporter protein (e.g., luciferase), so that its expression is controlled by the ability of the target analyte to interact with a specific regulatory proteins or receptor. The expressed reporter protein can be readily measured and directly related to the analyte concentration in the sample. Different recombinant yeast and bacterial biosensors were immobilized in a modified clear bottom black 384-well microplate to obtain a BL cell array suitable for on-site analysis. The cells array, which can be stored for up to 1 month at 4°C without loosing cell vitality, was characterized by high sensitivity (e.g., a LOD of 0.5 nM for testosterone was obtained with immobilized yeast cell-based biosensor for androgen detection after 1-month storage).
The performance of "contact" imaging is evaluated and compared to that of conventional optics-based imaging performed with laboratory instrumentation.

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ELECTROCHEMICAL AND PIEZOELECTRIC IMMUNOSENSORS FOR DETECTION OF BIOAGENTS

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Electrochemical sensors and piezoelectric quartz resonators were adopted as robust and sensitive transducers for construction of portable detection systems. The former approach utilized multichannel screen-printed sensors combined with specific antibodies and tracers based on peroxidase as a label [1]. The heterogeneous sandwich assay format used exchangeable sensors combined with an embedded flow system consisting of several miniperistaltic pumps. The piezoelectric immunosensor [2] provided direct measurement of the target bioagents, the sensing part was either used repeatedly after regeneration of the immobilized antibody or discarded in the case of gradually consumed binding capacity. From the construction point of view, both lines of portable detectors – ImmunoSMART and QCM-meter - shared several common elements (microcontroller, A/D converters, and pumps), though the sensing parts were different: multipotentiostat and oscillator-counter, custom flow-through cells and biosensors. Detectors were linked to the external computer with software LabTools (common data-management and user interface, individual detector-related plug-in modules).

The tested bioagents included the bacterium *Francisella tularensis* (*Ft*, belongs to cat. A biowarfare agents, though the LVS variant was employed) and model microbes *Escherichia coli* and *Bacillus atrophaeus*. The electrochemical system was able to detect *Ft* starting at 100 CFU/ml; one assay (incubations with sample and tracer, washing, addition of enzyme substrates, amperometric measurement) was completed within 20 min. The piezoelectric immunosensor with immobilized anti-*Ft* antibody indicated the presence of 10^5 CFU/ml directly within 10 min. Using the immunosensor modified with *Ft* derived lipopolysaccharides, rapid detection of anti-*Ft* antibodies from mice inoculated with the bioagent (0.1 LD50) was possible shortly (1-3 days) after infection. The *E. coli*-based bioaerosols were sampled after connection of the ImmunoSMART sensor with a cyclone device SASS 2300. These experiments were carried out in a closed chamber and 100 CFU/l in air was successfully detected.

The developed biosensor-based devices are promising tools suitable for rapid detection of bioagents as well as other microbial cells and biomolecules outside of laboratory.

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ELECTROCHEMICAL SENSORS, BIOSENSORS AND IMMUNOSENSORS APPLIED IN CLINICAL, FOOD AND ENVIRONMENTAL ANALYSIS.

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The scientific heredity left by Prof. Marco Mascini when he moved from Rome to Florence in 1985 has progressed in the field of electrochemical sensors and has produced several applications in the areas of clinical, food and environmental analysis. This keynote lecture will show some highlights during 25 years of research activity at the University of Rome Tor Vergata and is dedicated to Prof. Marco Mascini.

In these years we moved from the "first generation of biosensors" to "second" and "third" one, and from the use of classical sensors to the new, ease to use, cost effective, and versatile screen printed electrodes (SPEs) that resulted suitable for many modifications by the use of mediators and nanostructured materials. However, also "classical" potentiometric and amperometric sensors, constructed to micrometric scale, have been used for the detection of pH, calcium, potassium, ammonium and oxygen respectively to monitor the growth of cyanobacteria in the Roman Catacombs.

Prussian Blue (PB) modified SPEs have been assembled using a new chemical deposition procedure for the selective detection of hydrogen peroxide at low potential. The excellent analytical features of these sensors led to the development of a glucose biosensor that is now the analytical device of the Glucomenday, a new holter for continuous glucose detection in diabetic patients produced by the Menarini Company.

Another interesting application of the PB-based sensors is the possible detection of thiols in clinical samples. We have in fact demonstrated that the Prussian Blue not only acts as an electrocatalytic mediator for H_2O_2 reduction but also for the oxidation of several thiols. We have applied thiese sensors for the detection of thiocholine, a key analyte for the measurement of pesticides, heavy metals such as Hg, and glutathione. PB-SPEs were also applied to monitor hydrogen peroxide during organic reactions in ionic liquids, with the advantage of carrying out environmental friendly reactions, according to the principles of the "green chemistry".

Electrochemical disposable PB-modified biosensors based on cholinesterase enzyme inhibition have been assembled and used for organophosphorous and carbamic pesticides detection in river water samples and for the detection of chemical warfare agents (Sarin gas).

Concerning the use of nanomaterials, Carbon Nanotubes (CN) and Carbon Black (CB) nanoparticles have been used to assemble new sensors and biosensors able to measure, with improved sensitivity, important neurotransmitters such as dopamine, epinephrine and serotonin, biological molecules, as catechol, guanine and tyrosine, inorganic electroactive molecules as ferricyanide, and also hydrogen peroxide and NADH. Functionalized carbon nanofiber-based chemical sensors for the direct electrochemistry of the haemoglobin and myoglobin molecules have been developed. Electrochemical sensors modified with nanostructured polymers have been assembled for the detection of nitrites and ammonia to monitor the chemical potability of water. New nanomaterials such as few layer of functionalized graphene are actually under study for glucose biosensor applications.

SPEs have been also modified with films of Bi for the evaluation of lead bioaccumulation in aquatic macrophytic plant *Lemna Minor*. Also an exhaustive study of milk treatment to measure Pb in this complex matrix using bismuth modified SPE has been successfully carried out.

Immunosensors for marine toxins and for mycotoxins have been assembled and applied to detect the aflatoxin M1 in milk and the B1 in cereals. More recently trichothecenes DON, T-2, and HT-2 have been detected in cereals using electrochemical sensors arrays coupled with micromagnetic beads (as support for the immunological chain).

Immunosensors for different bacteria detection have been also developed. An interesting application in food analysis has been the assembling of an immunosensor for Salmonella detection

in different kind of meats with an analysis time of few hours instead of days. Immunosystems for the detection of Botulin toxins and for the rapid identifications of Celiac disease in patients are actually in progress in our research group.

A new research line concerns the development of new reagentless, single-step electrochemical DNA sensors (E-DNA). These biosensors have demonstrated to be able to recognize not only the complementary DNA sequence of the immobilized probe, but also other proteins such as anti-DNA antibodies, for example, directly in blood serum, permitting to track the progression of autoimmune diseases like systematic Lupus erythematosus, that affects more than five million people worldwide.

DEVELOPMENT OF NEW ELECTRONIC BIOSENSORS BASED ON ORGANIC THIN FILM TRANSISTORS

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Most of biological sensing techniques are based on optical detection principles that are highly sensitive and specific but very difficult to miniaturize. These techniques also require multiple reagents, long preparative steps and complex data analysis. Organic thin –film transistors (OTFTs) can offer an alternative to overcome some of the optical biosensors drawbacks. Simple and low-cost fabrication techniques, miniaturization, multi-parametric responses, signal amplification and label-free detection are the main features of OTFT biosensors[1,2]. Particularly, OTFT technology can be implemented to develop label-free DNA or bio-affinity sensor chips, having a field-effect transport directly coupled to a bio-sensing process, useful to high-throughput testing and point-of-care applications[3]. The development of new structures that can provide the full integration in OTFT devices of biological recognition elements such as antibodies or other proteins to confer specificity is the main goal of this study. The coupling of the OTFT device and the biological recognition system is actuated by assembling supramolecular structures that integrate biomolecules deposited directly in the OTFT active layer. Bio-probes are immobilized on the sensors surface using either conventional procedures and more innovative strategies based on the use of phospholipid bilayers. The efficiency of the immobilization procedures is evaluated by fluorescence imaging techniques. Preliminary results obtained by using the anti-biotin/biotin/streptavidine reagent systems will be presented. The possibility to develop OTFT biosensors capable of offering enhanced sensing performance, particularly in terms of sensitivity and bias control, will be also discussed. The reported technology hold the potential to display a great versatility allowing for a wide range of applicability to many different assays by just binding the right bio-recognition element for the analyte of interest. Besides, the label-free assay format ensures a reduction of assay time and reagents consumption with respect to methods relying on labeled molecules

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Au NANOPARTICLES IN ELECTROANALYSIS

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Metal nanoparticles (NPs) have recently assumed a prominent role in the development of amperometric sensors [1-4]. From a general point of view, NPs can be defined as the individual constituents of extremely fine powder (1-100 nm size), being usually considered transitional between the clusters, i.e. aggregates of few atoms, and the bulk scale. NPs possess a polyhedric shape, which makes a large fraction of atoms be located in the correspondence to such defects as vertexes and edges. This especially holds in the case of real NPs, typically under 20 nm size. Hence, metals under the form of NPs possess (electro)catalytic properties by far superior to those of bulk systems, which may increase the resolution of the response pattern, as well as the number of detectable/quantifiable analytes. Moreover, NPs possess a particularly high surface/volume ratio: when considering NP systems as electrode modifiers, an increased surface area is obtained with respect to bulk materials, often resulting in improved sensing sensitivity. Such a nanostructure may also increase the anchoring stability of elements sensitive toward species of biological interest and to facilitate the charge transfer.

This communication aims at critically summarising the most recent advances in the development of amperometric sensing systems based on AuNPs, ranging from chemical sensors for different inorganic and organic species to biosensors. Attention will be also devoted to the comparison of the synthetic approaches followed for obtaining nanostructured modified surfaces with the properties sought. The different techniques employed in the characterisation of nanostructured surfaces obtained after the deposition of AuNPs will be also outlined.

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BIOSENSORI ELETTROCHIMICI PER LA VALUTAZIONE DI ADDOTTI DE-BAP-DNA

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Lo studio della formazione di addotti stabili tra sequenze di DNA e composti organici è un'area sempre più importante della ricerca, soprattutto nel campo della verifica di tossicità di alcune molecole. L'interesse verso gli addotti del DNA dipende dal fatto che la produzione di queste sequenze disaccoppianti può dar luogo a processi di mutagenesi e carcinogenesi oltre a indicare l'esposizione del DNA al prodotto chimico in causa. La possibilità che un prodotto chimico, reattivo con il DNA, possa incrementare il rischio di cancro dipende da molti fattori e non necessariamente costituisce l'evidenza che il tumore apparirà nel tessuto.
In questa comunicazione saranno presentati dati relativi alla realizzazione di un biosensore elettrochimico a DNA per lo studio della formazione di addotti con un tossico modello. Come molecola modello è stato utilizzato il diolo epossido del benzo(a)pirene (DE-BaP) che è un metabolita del benzo-a-pirene ed è in grado di legarsi covalentemente al DNA tramite attacco nucleofilo e come sequenza target una sequenza oligonucleotidica di 24 basi appositamente disegnata inserendo 4 triplette hot-spot per la formazione di addotti con il DE-BaP.

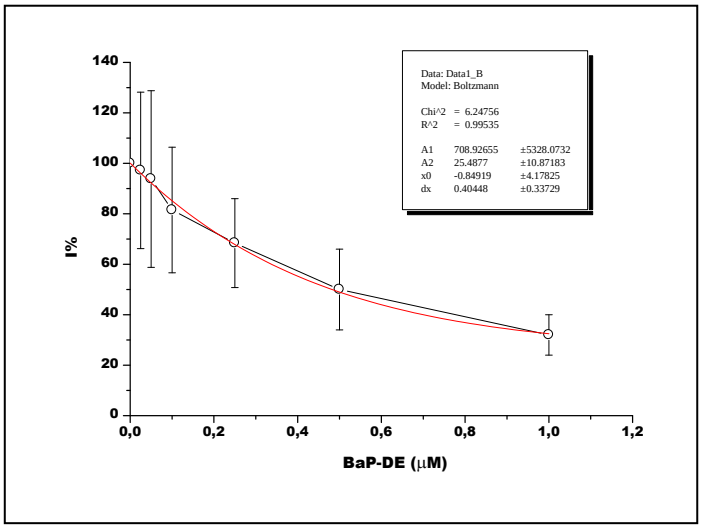

Figura 1: curva di calibrazione del DE-BaP ottenuta con il biosensore elettrochimico.

Il biosensore è stato ottenuto immobilizzando la sequenza oligonucleotidica, tramite un gruppo -SH terminale, sulla superficie d'oro di un elettrodo stampato. Il saggio per la verifica della formazione dell'addotto consiste nell'esporre la sequenza immobilizzata al composto tossico e successivamente, dopo uno step di lavaggio, il biosensore viene incubato con una soluzione contenente la sequenza oligonucleotidica complementare per la formazione dell'ibrido. Nel caso in cui sia avvenuta la formazione dell'addotto la reazione di ibridazione risulterà inibita, nel caso contrario si avrà la formazione dell'ibrido, Figura 1. Il meccanismo utilizzato per la verifica della reazione di ibridazione si basa sul sistema avidina-biotina utilizzando come marcatore enzimatico la fosfatasi alcalina con il substrato naftil-fosfato. La curva di calibrazione ottenuta mostra una dipendenza diretta tra quantità di tossico a cui viene esposta la sequenza oligonucleotidica target e l'inibizione dell'ibridazione.

L'USO DI PARTICELLE MAGNETICHE ED ELETTRODI SERIGRAFATI IN APTASENSORI NANOSTRUTTURATI DI AFFINITÀ PER LA DETERMINAZIONE DELLE MICOTOSSINE

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L'impiego di microparticelle magnetiche (MBs) che supportano l'immobilizzazione di aptameri per le prove impedisce competitivo adsorbimento aspecifico di tracciante coniugato e di altri bioreactive elettrodi stampati sulla superficie dello schermo in le fasi di lavaggio, di incubazione e affinità competitiva [1]. I MBs permettono anche il loro uso in applicazioni in cui anche stesso campione può causare interferenze a causa di adsorbimento sull'elettrodo.

Questo articolo descrive per la prima volta un aptasensor per la determinazione elettrochimica di ocratossina A (OTA) utilizzando elettrodi decorate e le procedure sperimentali molto simili a quelli utilizzati nel caso di immunosensori. Non vi è riportato aptasensor elettrochimico per la OTA, e alla nostra conoscenza non vi è aptasensor nessuna letteratura scientifica pubblicata per no altre micotossine. L'immobilizzazione di aptamero (biotinilato) sulla MBs e sulla base del forte legame avidita-streptavidina, e un meccanismo di generazione del segnale per l'uso di enzimi perossidasi. Il valore di EC_{50} (ng / mL) è di 1,2, e il limite rilevazione è 0,65 ng / mL di OTA.

Questo aptasensore è stato applicato in un campione certificato di farina di frumento (CRM B-MYC0880, Biopure, Tulin, Austria) con una tenore di OTA da 2.7 ± 0.1 ng /g, e un campione di farina è un certificato materiale di riferimento BCR-471 (IMMR, Commissione europea), un basso contenuto di OTA (< 0,6 ng / g, 95%). I tassi di recupero si trovano nell'intervallo circa tra 105-110%.

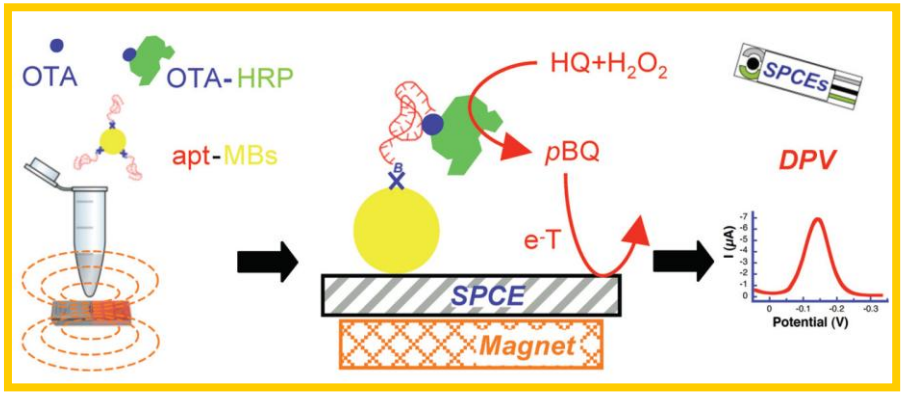

Figura 1. Schema del' aptasensor elettrochimico per la OTA.

Riferimenti

1. JC. Vidal, P. Duato, L. Bonel, JR. Castillo, *Analytical Chemistry, in press,* (2010)

Ringraziamo la società finanziaria *Aragonesa de Componentes Pasivos* (e il finanziamento PM 027/2007 Progetto ha ricevuto dal Governo di Aragona a 027-2007 il completamento di questo lavoro).

DNA-BASED BIOSENSOR, AND SURFACTANT TOXICITY: A FT-IR INVESTIGATION

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In the present work a DNA biosensor was used for the determination of the toxicity of different kinds of common surfactants.
The intensity of the guanine oxidation peak, measured through Square Wave Voltammetry usually quantifies (G% with respect to a buffer solution) the interactions between DNA and the eventually genotoxic substances. All surfactants caused a decrease of the guanine peak, in particular the nonionic surfactants were highly toxic (G% < 40%,) followed by the moderately toxic anionic surfactants (G% ~ 50) and cationic surfactants (G% ~ 55%). Some selected surfactants were investigated both in sea water and tap water, and data were compared to those obtained in acetate buffer. In several cases it was observed that toxicity decreases with increasing concentration as a consequence of surfactant self-assembly. Indeed, the self-assembly process competes with the interaction with DNA. Hence the toxicity can qualitatively be related with the self-assembly behavior of the different surfactants in the different media.
The interaction between surfactants and Calf Thymus DNA in solution and adsorbed on the sensor surface was investigated through FTIR-BioATR and FTIR-ATR spectroscopy respectively.

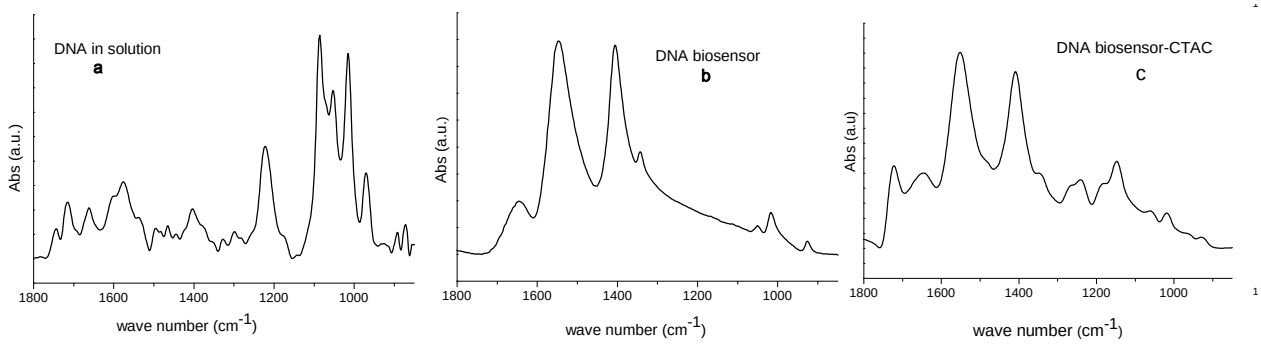

Figure 1. a) DNA spectra in solution; b) DNA biosensor spectra; c) DNA biosensor spectra after interaction with CTAC surfactant;

Significant specific modification of DNA bands appear as a result either of the immobilization process on the graphite solid surface or after the interaction with the surfactant as shown in figure1.

NON-INVASIVE PRENATAL DIAGNOSIS OF β-THALASSEMIA BASED ON FETAL DNA FROM PERIPHERAL BLOOD AND SEQUENCING OF β-GLOBIN PCR PRODUCTS

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We have determined the efficacy of sequencing PCR products obtained using as target free genomic DNA from peripheral blood enriched for low molecular weight DNA, which contains high quantity of free fetal DNA (ffDNA). Genomic DNA was isolated from peripheral blood of a pregnant woman exhibiting a β°39/β$^+$IVSI-110 genotype. After DNA isolation using QIAamp® DNA Blood Mini Kit (Qiagen), genomic DNA was separated by agarose gel electrophoresis, the low molecular weight DNA purified and PCR performed using primers amplifying a genomic β-globin DNA region containing the β°IVSI-1, β$^+$IVSI-6, β$^+$IVSI-110 and β°39 thalassemia mutations. The electropherograms obtained after sequencing of PCR products demonstrate that the samples enriched of ffDNA display a high β$^+$IVSI-110/wt peak ratio, while the β°39/wt ratio was low. On the contrary, the β$^+$IVSI-110/wt and β°39/wt peak ratios were found to be balanced in the PCR product from the maternal genomic DNA. This finding suggests a β$^+$IVSI-110/wt genotype of the fetus.

In order to determine the detection limit of the technology, DNA samples mimicking fetal DNA were diluted with maternal DNA and the same analysis performed. The results obtained suggest that the genotype of the ffDNA should be detectable when at least 15% of fetal DNA is present in the preparation.

Future perspectives of this approach should consider the combination between ffDNA isolation and other analytical techniques, including the use of Surface Plasmon Resonance (SPR)-based strategies. Our group has in fact demonstrated that SPR and Biospecific Interaction Analysis are efficient tools for detecting point mutation of β-thalassemia.[1,2]

Interestingly, recent developments of surface plasmon resonance technology, at least in theory, are compatible with the possibility to perform diagnostic analyses on PCR-free unamplified genomic DNA (D'Agata et al., manuscript in preparation), opening novel frontiers in molecular diagnosis, including non-invasive prenatal tests.

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SPRI FOR THE DETECTION OF GENOMIC DISORDERS IN UNAMPLIFIED HUMAN GENOMIC DNA

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DNA sensing is expected to be significantly improved by using simple and economic detection protocols which require minimal DNA modifications and provide enhanced signal amplification. In this perspective, the direct analysis of non amplified genomic DNA appears an excellent cost-effective alternative to the current available methods that can be achieved by using ultrasensitive DNA detection technologies.

Recently, we have shown that an ultrasensitive detection of non-amplified genomic DNA containing a target sequence as a minor component can be obtained by using a nanoparticle-enhanced Surface Plasmon Resonance Imaging (SPRI)-based detection protocol and surface-immobilized peptide nucleic acids (PNA) probes.[1,2]

In this work we demonstrate that point mutations in human genome can be also detected by adopting the nanoparticle-enhanced SPRI protocol based on the hybridization of sonicated non-amplified genomic DNA to PNA probes. The approach has been successfully implemented for the identification of the β°39 (C>T) thalassemia point mutation of the human beta-globin gene, demonstrating that the method provides a highly specific and cost-efficient approach for the detection of point mutations.

To the best of our knowledge, the method is the first reporting the possible use of SPRI in detecting human genetic mutations without PCR-mediated amplification. The observed detection efficiency allows us to claim that unamplified genomic DNA from low numbers of cells (few thousands) might be analyzed by using a very simple protocol, thus avoiding enzymatic treatment or complex thermal cycling of the genomic material. The efficiency is compatible with a PCR-free detection of human mutations starting with fetal-DNA enriched samples from peripheral blood in procedures aimed at the non-invasive prenatal diagnosis.

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ELECTROCHEMICAL GENOSENSOR ARRAY FOR COELEIAC DISEASE PREDISPOSITION ANALYSIS

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Coeliac disease (CD) is a small intestinal inflammation triggered by the intake of gluten, a protein present in certain cereals [1]; this has a prevalence of about 1% in the European population [2]. CD has been shown to affect only genetically predisposed individuals; strong relation between this disease and Human Leukocyte Antigens (HLA) has been proved, with 95% of the CD patients carrying HLA DQ2 genotype and ca. 3% carrying DQ8 genotype [3]. DQ2 and DQ8 negative individuals have been shown to be very unlikely to develop CD.
Electrochemical genosensors are low cost, easy to use, rapid, suitable for miniaturization and easy to integrate devices.
The aim of the reported work is the development of an electrochemical genosensors array for the rapid and cost effective typing of Coeliac disease associated HLA genes. The proposed sensing platform is based on the *Sequence Specific Oligonucleotides Probes (SSO) approach* where discrimination is made possible by the use of specific recognition probes. Electrochemical transduction of the probe/target hybridisation event is performed by the use of enzymatic label; this allowed a limit of detection of ca. 1 nM. ELONA, SPR and electrochemical evaluations of different probe designs, for the detection of CD associated alleles (DQA1*0201, DQA1*03, DQA1*050101/DQA1*0505, DQB1*02 and DQB1*03*), are reported. Optimal designs of probes for DQA1*0201 and DQA1*03 and DQA1*050101/DQA1*0505 has been achieved while further optimizations are required for DQB1*02 and DQB1*03* specific probes. Finally preliminary results on the analysis of PCR product will be also presented.

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A MIP-MODIFIED CARBON STRIP CELL AS SELECTIVE ELECTROCHEMICAL SENSOR FOR FIELD DETERMINATION OF TNT

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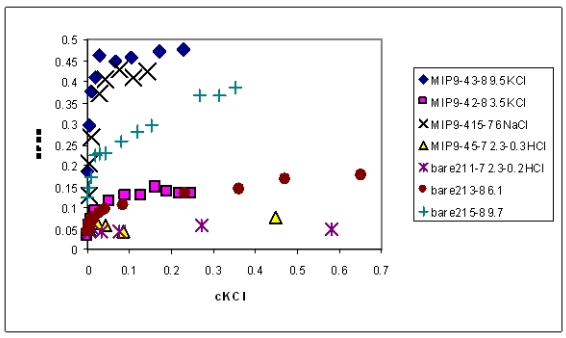
Aromatic nitroderivatives can be determined electrochemically since nitro groups are reduced at relatively high potentials. Several working electrodes have been proposed for the determination[1] included planar screen-printed carbon electrodes[2, 3]. These are very convenient devices for field determinations, particularly if integrated in strip cells consisting of planar electrodes, working, auxiliary and reference electrodes, all integrated in the same solid support. The resulting cell is miniaturized in comparison to the usual cells and is suitable for on site applications. Moreover very small solution volumes, even a drop of liquid, are required.

Strip cells can be prepared in a reproducible way and at low cost by the screen printing technology. For these reasons strip cells are suitable for in situ determinations of electroactive substances, for example heavy metals[4]. They have been used as working electrodes for detection of aromatic nitroderivatives[2, 3] and other organic electrochemically active substances. However, as usual in electrochemical methods, the poor selectivity can be a problem. In the sensor here proposed a good selectivity was achieved by cell modification with a thick layer of ion exchanging MIP.

A MIP (Molecular Imprinted Polymer) specific for trinitrotoluene (TNT) was obtained by the molecular imprinting technology methods. Methacrylic acid (MAA) was the functional monomer and ethylene glycol dimethacrylate (EGDMA) was at the same time the cross-linker and the solvent[5]. The liquid monomeric mixture was placed all over the zone of the strip containing the electrodes, and the polymerization was carried out at high temperature (80°C) The solid polymeric film formed in this way covers all three electrodes.

The electrolytic solution in contact with the cell is the MIP itself, in which the mobile cations are the counter ions of the negatively charged carboxylic groups fixed in the mainframe of the polymer. It has been found that the charge current is high in MIP-modified strip cell even in pure water, or in the air. Thus it is expected that measurements can be done directly in low ionic strength solution, as for example in ground waters.

The possibility of performing measurements without any treatment or modification of the sample was investigated, considering in particular the effect of acidity and ionic composition. Some results are reported in Figure 1, in which peak currents obtained at different conditions are reported.



It is seen that a signal is obtained at all the considered conditions, so that the presence of TNT can always be determined, even though different detection limits are attainable. The sensitivity is noticeably affected by the conditions.

Figure 1- ip (DPV) of 1 ppm TNT in μA on bare and MIP modified carbon electrode at different conditions

On the basis of the obtained results the conditions under which highest sensitivity was obtained were assessed.

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SOLID SUPPORTED MEMBRANES AS A BIOSENSOR SYSTEM FOR THE INVESTIGATION OF MEMBRANE TRANSPORTERS

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A biosensor technology, based on a solid supported membrane (SSM), is currently employed to detect charge movements in electrically active membrane transporters (1).
The SSM represents a model system for a lipid bilayer membrane with the additional benefit of being so mechanically stable that solutions may be rapidly exchange at the surface (2). Proteoliposomes or native membranes (vesicles or fragments) incorporating the transport protein can be adsorbed on a SSM and activated using a rapid substrate concentration jump. The substrate jump induces charge displacement within the transport protein, resulting in a current transient which can be detected in the external circuit (1,3). Therefore, the SSM serves two purposes at once, i.e. offering an adhesive surface to the adsorbed membrane entities and functioning as a transducer of a biosensor system.
An advantageous feature of the SSM method consists in providing an aqueous environment on both sides of the membrane for the incorporated transport proteins. In addition, adsorption of proteoliposomes or membranes allows a number of primary and secondary active membrane transporters to be immobilized on the SSM in a simple spontaneous process. Very recently, an automated version of the traditional SSM method has been developed which is adapted to the requirements of industrial drug screening (4,5).

The financial support of Ente Cassa di Risparmio di Firenze and Ministero dell'Istruzione, dell'Università e della Ricerca (PRIN Project) is gratefully acknowledged.

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INHIBITION OF MEMBRANE TRANSPORTERS: QUANTITATIVE INFORMATION OBTAINED THROUGH A BIOSENSOR TECHNOLOGY

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Membrane transporters are devoted to ion translocation through a lipid membrane phase. Among them, fundamental physiological roles are held by the ion pumps Ca-ATPase and Na,K-ATPase, prominent members of the P-type ATPases family (1).

BioElectroLab has a wide expertise in the study of ion transport by these proteins through a biosensor technology based on solid supported membranes (2). Our attention has been recently focused on the inhibition of ion pumps by molecules of potential pharmacological interest (3) and xenobiotics (4). Molecules like thapsigargin and cyclopiazonic acid belong to high (nanoM) affinity inhibitors of the Ca-ATPase, whereas curcumin and clotrimazole are medium (microM) affinity inhibitors of both Ca-ATPase and Na,K-ATPase. Moreover, the toxic heavy metal Pb^{2+}, that poses a major public health problem, is able to inhibit Na,K-ATPase activity in the low micromolar range. Frequently, inhibitors confine an ion pump in an inactive conformation.

Our experimental technique can provide useful information about the inhibition mechanism at molecular level, as well as quantitative data about the affinity of the molecule (or ion) for the enzyme through a half-inhibition constant value (K_i). Here we present a summary of results obtained in our laboratory for several compounds together with some preliminary data from the current research.

Financial support of Ente Cassa di Risparmio di Firenze and of MIUR (PRIN Project) is gratefully acknowledged.

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GOLD NANORODS FOR LSPR BASED BIOSENSORS

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Noble metal nanoparticles have attracted great attention due to their unique optical properties, in particular the localized surface plasmon resonance (LSPR). Because of the LSPR phenomenon, the nanoparticle extinction spectrum is affected by the particle size and shape as well as the constituting material and the surrounding dielectric media. Several analytical and bioanalysis applications, in which the nanorod optical properties are exploited, have been presented in the last years. Within them, various LSPR based biosensor formats have been proposed. The identification of an analyte could be accomplished by recording the shift of their LSPR peak. A specific bioreceptor is immobilised on the nanoparticle surface and the analyte/receptor biomolecular interaction modifies the dielectric properties of the surrounding medium, with consequent changes in the resonance peak.

In view of this, a study for the development of an immunosensor for anabolic androgenic steroids (AAS) based on the use of gold nanoparticles has been performed. In recent years the assumption of dietetic supplements and drugs is drastically increased because of current social and cultural habits. Most of these substances are included in the list of prohibited compounds of the World Anti-Doping Agency and within them the AAS. A World Anti-Doping Code has been drawn up in order to coordinate effective anti-doping programs. In this context, the demand of new rapid, efficient and throughput detection systems is always present.

In this work, first, a surface chemistry for conjugating antibodies specific for AAS to gold nanorods has been optimized and the obtained bioconjugates have been characterized. Moreover, the analytical performances of the bioconjugates are under evaluation in a capture competitive sensor format, in which the competitors are directly immobilised onto the sensor surface and the antigen-antibody recognition took place when the specific antibodies labelled with gold nanorods were introduced. Preliminary results demonstrated that the detection signal is due to the appearance of a plasmon peak only where specific antigen/antibody interaction occurs and that it is related to the different amounts of nanorods interacting with the surface.

In future, the identification of several prohibited substances can be accomplished by using bioconjugate nanoparticles differing in shape and size (label-encoded microarray) or by the location in the microarray (site-encoded microarray).

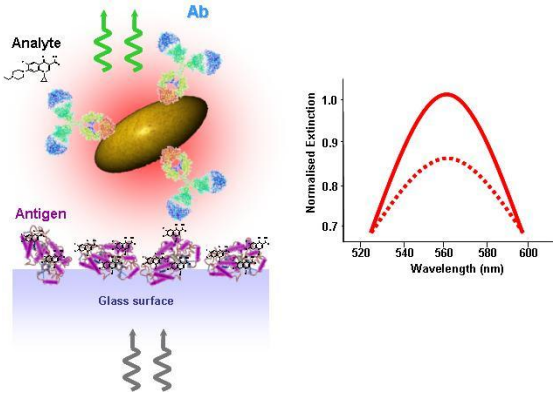

Figure 1. Gold nanorods addition over functionalized surface produces a change in the intensity of the plasmon peak.

BIOLUMINESCENT GENETICALLY ENGINEERED CELLS FOR BIOSENSING APPLICATIONS

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Bioluminescence (BL) has revealed an extremely useful analytical tool enabling ultrasensitive detection in biotechnological applications. Genetically engineered cells (bacteria, yeasts, or mammalian cells) able to produce a BL signal in response to a target analyte represent powerful bioanalytical tools for environmental, medical, and food analysis[1]. Their principal advantages are high sensitivity, rapidity, low cost, wide dynamic range, and adaptability to high-throughput screening.

Nevertheless some important issues have still to be addressed to make these biosensing systems true analytical biosensors, such as portability and reliability to enable their use for on field monitoring and point-of-care analysis.

To address this issue, a new polymeric matrix was developed to encapsulate and keep cells alive for long periods of time in order to obtain ready-to-use portable devices. Different recombinant yeast, bacterial and mammalian biosensors were immobilized to obtain a bioluminescent cell array that can be stored for up to 1 month at 4°C without loosing cell vitality[2]. These biosensing systems were obtained by introducing into bacterial or yeast cells a vector encoding for a receptor or a regulatory protein (e.g., human androgen/estrogen receptors, regulatory proteins involved in heavy metal-induced transcription…) and a vector with a BL protein (a luciferase) whose expression is controlled by activation of receptors or regulatory proteins. The expression of the reporter protein can be readily measured and directly related to the analyte bioavailability in the sample. The cell array is placed in contact, through an optical taper, with an imaging light sensor, a portable CCD camera able to localize and quantify the luminescent signal. Limit of detections comparable to those reported with liquid cultures were obtained with S.cerevisiae cells responding to androgens[3] and with different bacterial strains responding to heavy metals (cupper, zinc, lead) after 2 weeks storage of immobilized cells at 4°C (e.g., for the strain E. coli MC1061-cueR/pDNPcopAluc the LOD for cupper was 2×10^{-6} M with a dynamic range of 2×10^{-6} -2×10^{-3} M).

In addition, multiplexing capacity (high content screening) should be provided in order to reduce the number of analyses to obtain a complete sample profile. The recent availability of new reporter genes with improved BL properties, together with technical improvements, prompted the development of multiplexed cell-based assays using more BL proteins (e.g., green- and red-emitting luciferases, secreted luciferases or calcium sensitive photoprotein aequorin) under the regulation of constitutive and inducible promoters in the same cell. In particular multi-color bioassays were developed for the simultaneous monitoring of different targets (e.g., cyp3a4 and cyp7a1) together with cell vitality. This allowed to measure separate targets within the same cell with good precision (intra-assay and inter-assay CV below 13%).

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DEVELOPMENT AND VALIDATION OF AMPEROMETRIC IMMUNOSENSORS BASED ON NANOBIOCOMPOSITE MATERIALS FOR THE DETERMINATION OF ALPHA-FETOPROTEIN IN SERUM

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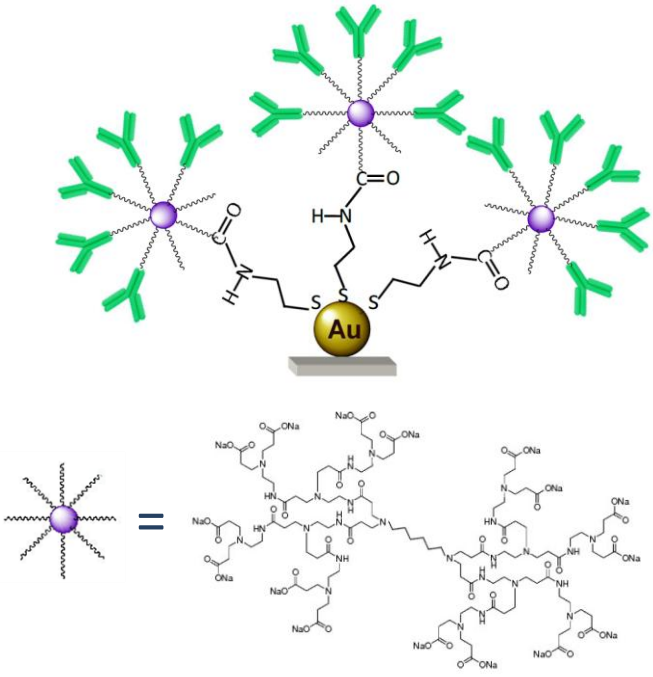
Immunosensors have become of special interest in several fields, one of their most important applications being the measurement of compounds of clinical or forensic interest in human serum. The present work deals with the development and validation of a simple to use and rugged immunosensor based on the working principle of the ELISA for the determination of alpha-fetoprotein (AFP) in serum. The peculiar properties of nanobiocomposite materials based on gold nanoparticles were exploited for the immobilization of the Antibody/Antigen/Antibody-HRP sandwich on a glassy carbon (GC) electrode surface to fabricate an amperometric biosensor.
The electrochemical deposition of nanogold on GC electrodes, associated with the use of monomeric thionin (redox mediator), dissolved in the reading solution allowed to provide a simple and reliable approach. Amperometric reading was performed by cyclic voltammetry (CV) measurements carried out with sandwich functionalized electrodes in a buffered solution (pH=6.8) of thionin acetate. Two-way analysis of variance (ANOVA) was applied to find the optimal concentration of the solutions used for the incubation of the antibodies.
The sensor was validated in serum assessing stability of the immunocomplex, linearity of response, limits of detection (3.7 ng/ml) and quantitation (11 ng/ml), precision (intra- and inter-sensor repeatability) and recovery rate (103%).The stability of the GC/Ab functionalized substrate was demonstrated over one month, showing variation coefficients below 5%.
Experiments with real samples of clinical interest were also carried out[1].



Studies aimed to the amplification of the response by means of dendrimer-based linking of the catching antibody were also carried out showing interesting findings.
For this purpose a self-assembled monolayer of 2-aminoethanethiol (AET) was deposed onto nanogold, and PAMAM G1.5 dendrimers were then bound to the linked AET.
Preliminary results obtained with dendrimer-linked antibody show an improvement of the performance in terms of reduced blank signal and widened dynamic range of response

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Design razionale di sonde da utilizzare in sensori a DNA: un approccio computazionale con verifica sperimentale

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Nello sviluppo di un biosensore a DNA la scelta di recettori appropriati per rivelare l'analita in esame, rappresenta un passo fondamentale per garantire l'efficacia della misura. Diversi aspetti vengono normalmente considerati nello scegliere la sequenza delle sonde, tra cui in primo luogo la selettività del recettore verso il target, la lunghezza, il contenuto in GC. In ogni caso questi sono solo alcuni dei fattori che caratterizzano l'efficacia delle sonde immobilizzate sul biosensore. Nasce quindi la necessità di implementare i metodi per la selezione delle sonde, cercando di sviluppare un metodo per un design razionale dei recettori che dia la possibilità di valutare le performance analitiche di una sonda *ex ante*, prima di testarla dal punto di vista sperimentale, evitando sprechi pecuniari e di tempo. Questo lavoro è volto a sviluppare un approccio basato sulla selezione *in silico* delle sonde che tenga conto dei parametri importanti nelle scelta delle sonde e che possa essere poi utilizzato in sensori a DNA, indipendentemente dal principio di trasduzione.
Le caratteristiche dei recettori sono state valutate *in silico* quindi usando un appropriato programma sviluppato da *Wernersson et al.* (OligoWiz [1]). Data la sequenza target, il software è capace di assegnare ad ogni possibile sonda specifica per quell'analita, un punteggio che riflette la bontà del recettore stesso. Il punteggio viene assegnato in base a dei calcoli che sfruttano l'algoritmo “nearest neighbor”, cioè valutazioni computazionali che tengono presente l'influenza che i vari nucleotidi di una sequenza hanno l'uno sull'altro.
Per validare il metodo proposto abbiamo selezionato tra tutte le possibili sonde per un target modello, un set di cinque sonde con differente stimata, *in silico*, capacità di riconoscimento. L'approccio teorico è stato testato sperimentalmente su un biosensore ottico basato sulla risonanza plasmonica di superficie per immagini (SPR-i). L'andamento dei segnali SPR per le diverse sonde rispetta il trend dei diversi punteggi assegnati dal programma. I dati sperimentali quindi confermano che le predizioni computazionali riflettono realmente l'efficacia delle sonde immobilizzate sul biosensore nel legare il target d'interesse.

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APPLICAZIONI DI ELETTRODI MODIFICATI IN LINGUE ELETTRONICHE PER L'ANALISI CIECA DI MATRICI ALIMENTARI

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Le lingue elettroniche (ET) rappresentano uno strumento analitico emergente che può essere usato sia per l'analisi compositiva di matrici alimentari che per una rapida valutazione della loro qualità, richiedendo un ridotto o addirittura nullo preliminare trattamento del campione. Una ET comprende un set di sensori con parziale selettività chimica e specificità solo incidentale, accoppiato ad un opportuno sistema di pattern recognition per il trattamento dei dati. Il segnale proveniente dai sensori, ad elevato contenuto informativo, viene trattato con i tipici approcci dell''analisi cieca', volendo cioè ottenere una sorta di 'impronta digitale' del campione analizzato, piuttosto che una dettagliata composizione quali- e quantitativa dello stesso. In questo caso, l'effetto matrice, che costituisce un problema nell'analisi chimica, può essere una fonte aggiuntiva di informazione utile. In linea di principio, questo approccio consente di rispondere ad esigenze quali il riconoscimento e la classificazione di campioni, il monitoraggio di processo, l'analisi qualitativa e la valutazione delle caratteristiche sensoriali. Inoltre, attraverso tecniche di calibrazione multivariata, possono essere possibili anche determinazioni quantitative specifiche. Array di sensori elettrochimici hanno dimostrato di essere altamente efficienti nella discriminazione di matrici complesse liquide. In particolare, sensori voltammetrici presentano numerosi vantaggi dovuti alla loro alta versatilità, sensibilità e semplicità, nonché alla possibilità di modificare la superficie elettrodica con materiali diversi, aumentando ulteriormente sensibilità e selettività e riducendo fenomeni di passivazione.

In questo contesto, abbiamo recentemente dimostrato i vantaggi offerti dall'uso di polimeri conduttori come modificanti della superficie elettrodica in ET per il riconoscimento di diversi succhi di frutta e per la discriminazione di vini bianchi e rossi rispetto a varietà e provenienza geografica [1-3]. In particolare, abbiamo verificato l'efficacia di uno specifico sensore, un elettrodo di Pt ricoperto da un film di poli-etilendiossitiofene (PEDOT), accoppiato ad un'opportuna trattazione chemiometrica basata su tecniche di analisi multivariata. Sulla base di questi risultati, abbiamo esteso lo studio dell'efficienza di questo sensore ad un più ampio numero di situazioni, e, come obiettivo più ambizioso, alla quantificazione di alcuni parametri chimico-fisici di particolare importanza nel settore enologico. Nella presente comunicazione, riportiamo i risultati ottenuti usando elettrodi convenzionali e microelettrodi modificati da PEDOT per la valutazione di alcuni parametrici significativi per il controllo di qualità di vini rossi, quali la SO_2, i polifenoli totali e l'indice di colore. Essi sono comunemente valutati mediante analisi chimiche convenzionali che richiedono spesso tempi lunghi e pretrattamento del campione, se non distruzione dello stesso. Accanto alla possibilità di costruire modelli di calibrazione, abbiamo testato la capacità discriminante del sistema da un punto di vista qualitativo (riconoscimento dei campioni di vino analizzati in base a varietà ed origine) e quantitativo (identificazione dei campioni aventi parametri eccedenti valori di soglia significativi). L'uso di un elevato numero di campioni consente di esplorare un ampio intervallo di valori dei parametri chimico-fisici considerati, dando particolare significato sia al sistema di rilevazione che al trattamento chemiometrico dei dati. La competitività del sistema messo a punto può essere valutata anche considerando la possibilità di applicazione in procedure rapide di pre-screening in controlli di routine, nonché in analisi effettuate online.

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ELECTROCHEMICAL BIOSENSORS COUPLED TO MAGNETIC BEADS FOR THE DETECTION OF CLINICAL BIOMARKERS

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Magnetic beads are known to be a powerful tool in a variety of bioassays. Their use improves the performances of the affinity interaction for the faster assay kinetics achieved because the beads are in suspension and for the minimized matrix effect due to improved washing and separation. Moreover, they allow the analysis of complex samples without any pre-enrichment or purification steps.

This work deals with the use of magnetic beads as solid support for the immobilization of several bioreceptors: from antibodies to aptamers, passing through nucleic acid strands. Different bioassays have been developed and applied to the detection of clinical biomarkers. Thus, the development of an electrochemical immunoassay for the detection of inflammatory marker, such as neopterin, is presented. Application of aptamers specific for clinical biomarkers such as thrombin and C-reactive protein (CRP), is also discussed[1]. Different assay formats (sandwich, direct and indirect competitive scheme) have been performed using magnetic beads as solid phase and carbon screen-printed electrodes as transducers. In all cases an enzymatic conjugate (streptavidin-alkaline phosphatase) has been used for the evaluation of the extent of the molecular recognition, α-naphthyl phosphate as enzymatic substrate and Differential Pulse Voltammetry (DPV) for the detection of the enzymatic product. Moreover, magnetic beads coupled to electrochemical transduction have been used to detect tumor marker through the evaluation of the hybridization reaction[2]. For this application specific nucleic acid strands have been immobilized onto the magnetic beads and applied to the detection of complementary DNA or RNA sequences considered as tumor marker. The possibility to integrate the detection scheme in a microfluidic platform is also presented[3]. For this purpose a microfluidic device combining a special chip containing eight polymer microchannels, with a portable, computer-controlled instrument has been used. The presence of micro-electrodes in each channel allows the direct quantification of the affinity reaction by conducting eight in-parallel electrochemical measurements.

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POSTERS

ELECTROCHEMICAL APTAMER-BASED BIOSENSOR COUPLED TO PARAMAGNETIC MICROPARTICLES FOR TOBRAMYCIN DETECTION

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Tobramycin is a broad-spectrum aminoglycoside antibiotic which exhibits bactericidal activity against some *Gram-positive* and many *Gram-negative* organisms. Like other aminoglycosides, tobramycin shows a narrow therapeutic range (2-12 μg/mL in serum) owing to potential side adverse effects such as oto- and nephrotoxicity. Therefore, careful monitoring of the drug levels in patients serum is required. The lack of electrochemical and spectroscopic properties makes the development of methods of analysis for tobramycin a challenging task.

For this purpose, we propose the employment of an electrochemical aptasensor taking advantage of the specific recognition of an RNA anti-tobramycin aptamer[1], which was modified at 2´ ribose position with a –OMe group to increase its nuclease resistance. Carboxylated paramagnetic microparticles were chosen as an adequate sensing phase platform because of the simplicity of handling and modification protocol, in this particular case with molecules containing amino groups, as tobramycin, via carbodiimide chemistry. The electrochemical measurements were run on screen-printed carbon electrochemical cells (SPCE).

The proposed assay is based on a general format assay for small molecules determination previously reported[2] and consists of an inhibition assay. The sample is incubated with an optimized fixed concentration of 5´-biotinylated anti-tobramycin aptamer (BATA), 0.1 μM. Afterwards modified beads are exposed to this solution. This way, the free aptamer will bind to the tobramycin linked to the magnetic particles. Then, the streptavidin-alkaline phosphatase ($Strep_2$-AP) conjugate is added, and finally, the addition of the substrate (α-naphthyl phosphate) allows the electrochemical detection of the product enzymatically generated, α-naphthol, by Differential Pulse Voltammetric (DPV) measurements.

The signal measured (expressed in percentage, S%, of the maximum signal which corresponds to the blank) decreases with a linear pattern with the tobramycin concentration. The sensing surface was evaluated in terms of its reproducibility, detection limit, dynamic range and selectivity towards other aminoglycosides antibiotics.

Acknowledgments

E.G.F. and N.S.A, thank to Spanish Government for a predoctoral grant and a Ramón y Cajal contract respectively. This work has been co- financed by Projects CTQ2008-02429 and FICYT IB08-87 and the European Regional Development Fund.

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STUDY OF THE COMBINATION OF THE DEPOSITON/STRIPPING OF SACRIFICIAL METALNANO-STRUCTURES AND ALKANE THIOL SELF ASSEMBLING AS A ROUTE FOR GENOSENSOR SURFACE PREPARATION

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In the reported work the electrodeposition/stripping of metallic sacrificial nano-structures in combination with self-assembled alkane thiols have been investigated as a route of generating randomly nano-structured surfaces for electrochemical genosensor application (Figure 1).

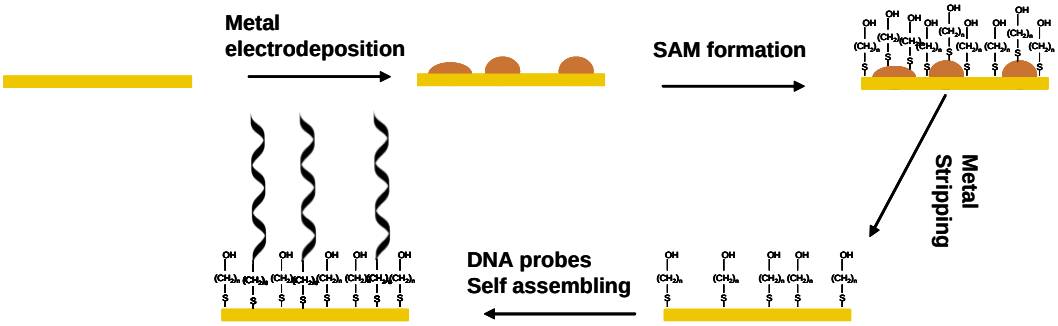

Figure 1: Scheme of the proposed surface preparation protocol.

In order to accomplish the proposed target several aspects have been investigated including: (i) metal electrodeposition, (ii) alkane thiol SAM formation on different metals, (iii) metal stripping from SAM modified surfaces, (iv) nano-structuring, (v) probe immobilisation and evaluation of sensor performance. Electrodeposition of the sacrificial metal (Cu) on Au electrode was performed using a three step chrono-potentiometry process in order to promote nano-structure formation, confirmed by AFM imaging, instead of metal underpotential deposition. Evaluation of the Cu stripping, as a function of the presence of alkane thiol monolayer, showed an increase in stripping potential with the length of the alkane thiol. Formation of vacancies in the alkane thiol monolayer was demonstrated by studying the self assembling of thiol modified ferrocene; this occurred only when the stripping step was performed on a sensor with previously electrodeposited the sacrificial metal. Finally the prepared surface was tested as platform for the assembling of thiol modified probes in the preparation of genosensor. Suitability of the prepared surface for sensing application was tested by amperometric detection of Cystic Fibrosis associated mutation (DF508).

USING OF INDIGO CARMINE AS ELECTROACTIVE INDICATOR FOR DETECTION OF SHORT SEQUENCE OF P53 GENE BY PNA HYBEIDIZATION BIOSENSOR

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Over the last decades, many methods have been developed for the electrochemical detection and analysis of DNA. The analysis of DNA is one of the key health issues facing our society today and is applied in monitoring of gen expression, in forensic, in pharmaceutical applications, consumer products and food control. In this work, a new electrochemical PNA hybridization biosensor for detection of a 15-mer sequence unique to p53 using indigo carmine (IC) as an electrochemical detector is described. IC is a dye which occurs as a dusky, purplish-blue powder or blue granules having a coppery luster. In recent years, IC has been applied by some researchers in various fields such as spectrophotometry and electrochemistry [1-4]. This genosensor rely on the hybridization of the oligonucleotides target with its complementary probe which is immobilized on gold electrode by self-assembled monolayer. Because this label is electroactive in acidic medium, the interaction between IC and short sequence of p53 is studied by differential pulse voltammety (DPV) in 0.1M H_2SO_4. The results of electrochemical impedance spectroscopy data and cyclic voltammetry in $[Fe(CN)_6]^{3-/4-}$ redox solution show no breakage in PNA-DNA duplex. Decline in the voltammetric peak currents of IC is observed upon hybridization of the probe with the target DNA (Fig.1).The influence of probe concentration on effective discrimination against noncomplementary oligonucleotides is investigated and 10^{-7} M is obtained as optimum concentration for immobilization of probe on the electrode. Diagnostic performance of the PNA sensor is described and the detection limit was found to be 9.8×10^{-12} M.

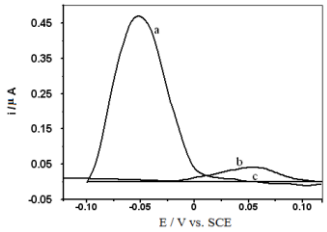

Fig. 1. Differential pulse voltammogram of accumulated IC at bare Au electrode (carve a), at 10^{-6} M PNA probe modified Au electrode before hybridization (carve b) and after hybridization with 10^{-6} M target DNA (carve c) in 0.1 M H_2SO_4 solution, Pulse height: 0.025 V, scan rate: 100 mV s^{-1}.

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ELECTROCHEMICAL AND SPECTROSCOPIC INVESTIGATION OF INTERACTION OF Yb^{3+} IONS WITH SHORT SINGLE STRAND DNA SEQUENCE FOR BETTER UNDERSTANDING OF Yb^{3+}–ssDNA COMPLEXES

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The binding of cations to DNA can be an interesting field of research due to the importance of these ions in biological media. These counter ions and interactions are influence on the conformations and interactions of a DNA. With DNA, two modes of ion interaction have been distinguished: purely electrostatic, and coordinate to phosphate oxygen or to bases [1].

In this work, for the first time the electrochemical behavior of the Tb^{3+} and Tb^{3+} ion interaction with short single strand DNA (ssDNA) sequence in two pHs was studied. Then the UV-Vis spectroscopic method was used for supporting these evidences. The ratio between [Tb^{3+}] and [ssDNA] is dependence to pH and pK_a of DNA bases. In pH 3.7, Tb^{3+} binds to ssDNA mainly by electrostatic attraction [2]. Binding number, n, of 1 of Tb^{3+} per ssDNA and binding constant, β_s, were obtained with cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods, respectively (Fig. 1) [2]. The UV-Vis study showed similar results. The results in pH 5.5 show that Tb^{3+} can bind to ssDNA with electrostatic and covalent bond. The binding number 2 of Tb^{3+} per ssDNA was obtained. Computational studies were done and confirm the result of experimental data. The agreement mutually verifies the accuracy of the methods.

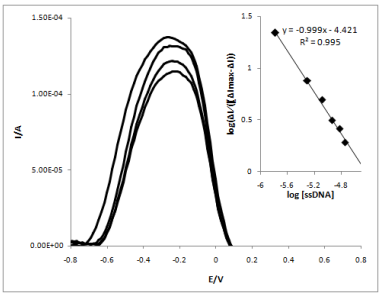

Fig. 1 Differential pulse voltammograms in different concentration (a) 5×10^{-4} M Tb^{3+}, (b) a + 1.67×10^{-6} M ssDNA, (c) a + 5.0×10^{-6} M ssDNA, (d) a + 1.16×10^{-5} M ssDNA, in pH=3.7, initial potential, -0.8 V, the end potential, 0.1 V, the step potential, 0.015 V, the modulation time, 0.02 s, the interval time, 0.53 s.

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QUENCHING EFFECTS OF SOME AMINO ACIDS ON PEROXYOXALATE CHEMILUMINESCENCE OF RUBRENE

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The brilliant emissions resulting from the oxidation of certain oxalic acid derivatives, especially in the presence of a variety of fluorophores, are the bases of the most active area of current interest in chemiluminescence [1, 2]. The quenching effect of amino acids L-cysteine, L-methionine and L-histidine on strong chemiluminescence of bis(2,4,6- trichlorophenyl)oxalate–H_2O_2 system in the presence of Rubrene (5, 6, 11, 12-tetraphenylnaphthacene) was studied. Kinetic parameters for the peroxyoxalate-chemiluminescence (PO-CL) were also calculated from the computer fitting of the corresponding chemiluminescence intensity/time profiles. These systems resulted in Stern–Volmer plots in the quencher concentration range of 2. 5×10^{-5} to 2.25×10^{-4} M, with K_Q values of 2.3×10^4, 1.3×10^4 M^{-1} and 0.8×10^4 M^{-1} for L-cysteine, L-metionine and L-histidine respectively. The mechanism of PO-CL process has been postulated to involve at least one highly energetic intermediate (possibly a dioxetane species) capable of exciting a fluorescent receptor molecule [3,4].

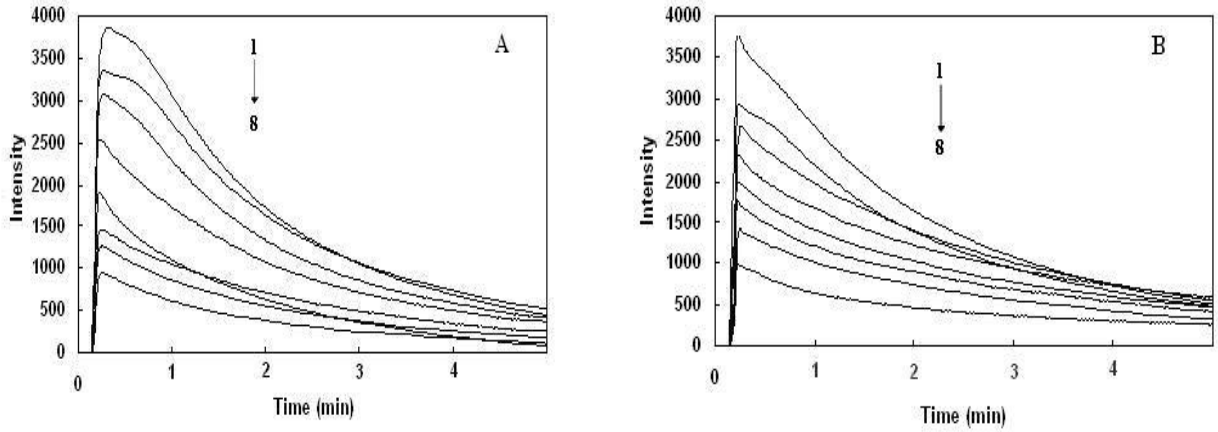

Fig. 1 A . CL-intensity as a function of time for the H_2O_2–TCPO– Rubrene system in the absence and presence of varying concentration of Met: (1) 0.00 M, (2) 2.50×10^{-5} M, (3) 5.00×10^{-5} M, (4) 7.50×10^{-5} M, (5) 1.00×10^{-4} M, (6) 1.50×10^{-4} M, (7) 1.75×10^{-4} M, and (8) 2.25×10^{-4} M. 1 B. CL-intensity as a function of time for the H2O2–TCPO– Rubrene system in the absence and presence of varying concentration of His same as Met.

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SELECTIVE VOLTAMMETRIC DETERMINATION OF ELECTROACTIVE NEUROMODULATING SPECIES IN BIOLOGICAL SAMPLES USING CARBON NANOTUBE MODIFIED WORKING ELECTRODE

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As it is well known, among molecules taking part in complex biochemical reactions several are electroactive. Measuring their concentration is important task in clinical diagnosis as well as in in different areas of experimental life sciences. Monoamines, dopamine (DA), norepinephrine (NE), serotonin (5-HT) and their oxidative metabolites are involved in propagation, or modulation of the propagation of neural information in the neural system of animals. There have been high numbers of methods developed for determination of these species in biological samples. Most of these are using chromatographic separation of specially prepared, pre-treated samples. Some of these inverse phase HPLC methods use electrochemical detection. Carbon nanotubes (CNTs) working electrodes have been proved beneficial helping the electron exchange reaction in redox processes of different species. In this way the performance of different voltammetric measuring methods could be considerable improved. In our recent work the performance of a CNT modified electrode with ferophthalocyanine (FePc) have been investigated aiming to work out well applicable method for the analysis of monoamine neurotransmitters in small volume biological samples. Voltammetring test methods as well as methods of surface characterizations have been employed in the studies. The effects of different interfering species appearing in biologic samples have been investigated. The selectivity and the lower limit of determination could be improved by application of nanotube modification. The monoamine neurotransmitter measuring method worked out has been tested in analyzing real clinical samples of a few microlitre volumes. Our presentation will summarize our new findings and the proposed analytical protocol worked out.

ELECTROCATALYTIC VOLTAMMETRIC DETERMINATION OF GUANINE AT A COBALT PHTHALOCYANINE MODIFIED CARBON NANOTUBES PASTE ELECTRODE

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A novel assay for the electrochemical detection of guanine based on carbon nanotubes paste electrodes (CNTPEs) modified with cobalt phthalocyanine (CoPc) has been investigated. The results indicated that the modification of a CNTPE with this compound results in amplification of the guanine oxidation response in contrast to that on the unmodified CNTPE. The electrochemical behavior of the modified electrode and the mechanism of the oxidation of guanine and ssDNA were investigated using cyclic (CV) and differential pulse voltammetry (DPV). The methods parameters were optimized. Detection limits of 1.3×10^{-7} moles·L^{-1} and 9.86×10^{-8} moles·L^{-1} were obtained for guanine and ssDNA, respectively, by using the electrocatalytic oxidation signal corresponding to the Co(II)/Co(III) redox process. The advantages of convenient fabrication, low-cost detection, short analysis time and combination with nanotechnology for increasing the sensitivity make the modified electrodes worthy of special emphasis in the nonlabeled detection of DNA hybridization reaction and in the development of DNA based biosensors for toxic chemicals, toxins and pathogens determination.

IMMOBILIZATION OF SINGLE-STRANDED DNA ONTO BORON DOPED DIAMOND ELECTRODE

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The synthesized boron doped diamond (BDD) is hydrophobic and the surface terminations are mainly C–H functions. This surface is sensitive to anodic polarization in aqueous media and allows partial derivatization of C–H terminations into carbonyl, carboxyl and hydroxyl functions [1,2]. The BDD electrochemical oxidation leads to enlargement of the electrochemical window in aqueous media specially in oxidation case [2] and it is useful for the electrochemical determination of highly positive oxidation species such as purine (guanine and adenine) and pyrimidine (cytosine and tymine) bases. Moreover, the carboxyl terminations on the BDD electrode seem to be an interesting coupling method of DNA.

In this work, the activated (by EDC/NHS) carboxyl groups on the BDD electrode were used as platform for streptavidin (STA) immobilization and then biotinylated single-stranded DNA (5'-CGCTCAATGCCTGGAGAT-3') attaching.

Cyclic voltammograms of the $K_4Fe(CN)_6$ in 0.1 mol L^{-1} phosphate buffer solution (pH 7.0) were carried out on the H-terminated BDD surface (electrode pre-treatment at +3.0 and –3.0 V during 5 and 30 s respectively in a 0.5 mol L^{-1} H_2SO_4 solution) and on the carboxyl terminated BDD surface (electrode pre-treatment at +3.0 V for 60 min in a 1 mol L^{-1} H_2SO_4 solution). The kinetics and voltammetric response of the $Fe(CN)_6^{4-/3-}$ redox couple were greatly affected by the carboxyl groups on the BDD surface.

The response of DNA mononucleotides guanosine (GMP), adenosine (AMP), thymidine (TMP) and cytidine 5'-monophosphate (CMP) in solution (individually and in a mixture) were evaluated by square wave voltammetry on the BDD-COOH. The baseline corrected voltammograms for individual nucleotides presented satisfactory responses at 1.14, 1.49, 1.75 and 1.86 V *vs.* Ag/AgCl, for GMP, AMP, TMP and CMP, respectively. The peaks of thymidine and cytidine overlapped in an equimolar mixture of all analytes.

The carboxyl terminated BDD surface was activated by EDC and NHS (3.1 and 1.7 x 10^{-2} mol L^{-1} for 1 h). After this, 0.1 mg mL^{-1} streptavidin protein was immobilized on the BDD-COOH (1h incubation time). Sequently, 10 µg mL^{-1} ss-DNA was attached on the BDD-COO-STA for 1 h. Square wave voltammograms were performed in phosphate buffer solution (pH 7.0) for BDD-COOH (1) and BDD-COO-STA-DNA (2). The voltammogram 1 was subtracted from the voltammogram (2) and presented two peaks. The first peak at 1.16 V refers to guanosine oxidation and a large peak at 1.78 V *vs.* Ag/AgCl possibly refers to a mixture of adenosine, thymidine and citosine response. Peaks of the nucleotides evidence the immobilization of ss-DNA on the BDD-COOH.

The results showed the possibility of ss-DNA immobilization on the carboxyl terminated BDD electrode which can be used for evaluating the interaction of substances with DNA or hybridization reactions. It is worth mentioning that the pyrimidines oxidation is hardly observed in glassy carbon, carbon paste and graphite electrodes but are evident on BDD-COOH surface.

Acknowledgements: to FAPESP, Brazil, for financial support.

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SVILUPPO DI UN IMMUNOSISTEMA ELIME PER LA DETERMINAZIONE RAPIDA DELLA CELIACHIA

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Il morbo celiaco è una delle malattie immunomediate più comuni nei paesi occidentali con un importante problema di sottostima del numero di persone realmente affette dovuta ad una mancata o non corretta diagnosi (effetto iceberg). Per questo, la comunità scientifica pone l'accento sulla necessità di una diagnosi precoce per prevenire lo sviluppo di forme patologiche più gravi.
Attualmente il principale esame di laboratorio consiste nel dosaggio sierico degli anticorpi IgA anti-transglutaminasi tissutale (tTG) tramite sistemi immunoenzimatici di tipo ELISA.
Scopo di questo lavoro è lo sviluppo di un immunosistema elettrochimico per la determinazione delle IgA anti-tTG che possa essere applicato sia sul classico campione di siero che su campioni di saliva, rendendo l'analisi la meno invasiva possibile. In questo modo il sistema potrà essere utilizzato per diagnosi preliminari di screening, anche nel "doctor office" e/o in strutture non ospedaliere, diventando un "point of care testing" (POCT). Il sistema realizzato è di tipo ELIME (Enzyme-Linked ImmunoMagnetic Electrochemical assay), basato sull'utilizzo di particelle magnetiche, con immobilizzato l'antigene tTG umana, accoppiate con strisce magnetizzate di 8 elettrodi Screen Printed (SPE).
Risultati principali ottenuti:
-sviluppo dell'immunosistema a rilevazione elettrochimica dotato di elevata sensibilità analitica e rapidità (tempo d'analisi ~ 30 minuti);
-valutazione dell'esattezza e della precisione del metodo mediante analisi di sieri bianchi (provenienti da pazienti non celiaci), addizionati con concentrazioni note di IgA anti-tTG;
-validazione del sistema tramite misura di campioni di siero e realizzazione della curva ROC per la determinazione del cut-off ottimale (2 UA/ml) con il quale sono state ottenute una sensibilità clinica del 94.74% ed una specificità clinica del 100%.
Sono in corso prove per poter adattare il sistema sviluppato all'analisi di campioni di saliva.

L'attività di ricerca è stata svolta in collaborazione con RADIM SpA nell'ambito del "Progetto di Ricerca Industriale e Sviluppo Sperimentale Filas 2008: Biosensori per la determinazione rapida (POCT) della celiachia e delle allergie".

VERTICALLY ALIGNED POLYANILINE NANOTUBES FOR CHEMICAL GAS SENSING

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The development of chemical sensors based on single nanostructures with reliable electrical contact is currently a challenging topic in materials research. Different approaches have been reported in the literature to contact nanostructures, such as the use of Electron Beam Lithography or a Focused Ion Beam for metal contact deposition over nanostructures. However, all of these techniques are extremely complex and expensive and do not always ensure a stable and durable electrical contact.
In this work, we synthesised vertically aligned polyaniline (PANI) nanotubes, directly connected to double electrical contact, via electropolymerisation of a novel monomer, N-phenylethylene diamine methacrylamide (NPEDMA), using an alumina membrane as a template (see Figure1,a).

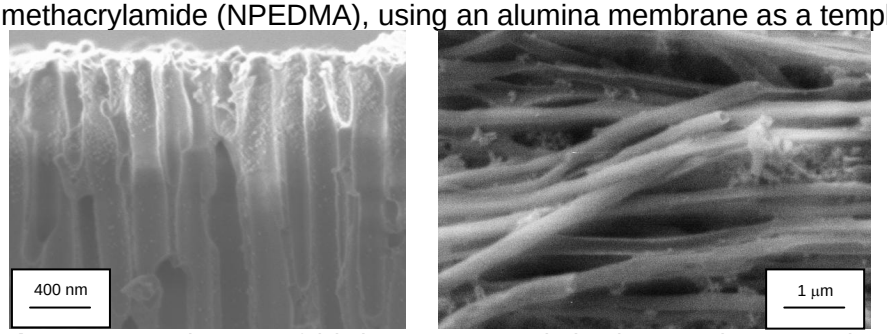

Figure 1. SEM images of (a) the Au sputtered alumina membrane used as a template, cross-sectional view; (b) a bundle of PANI nanotubes after membrane dissolution.

These structures were characterised using SEM (Scanning Electron Microscopy) (see Figure 1,b), EDX (Energy Dispersive X-ray) analysis and STEM (Scanning Transmission Electron Microscopy). The nanotubes were then functionally characterised as sensors by exploiting their chemical interaction with gases. Resistance variation of the polymer in presence of different gases (NO_2 - see Figure2, ethanol, NH_3 and CO) was exploited to develop an electrical gas sensor. Moreover, the possibility to develop an optical gas sensor was also evaluated: fotoluminescence emission of the polymer in presence of NO_2 was measured. Both approaches offer important possibilities for applications in biosensing and the construction of biosensors.

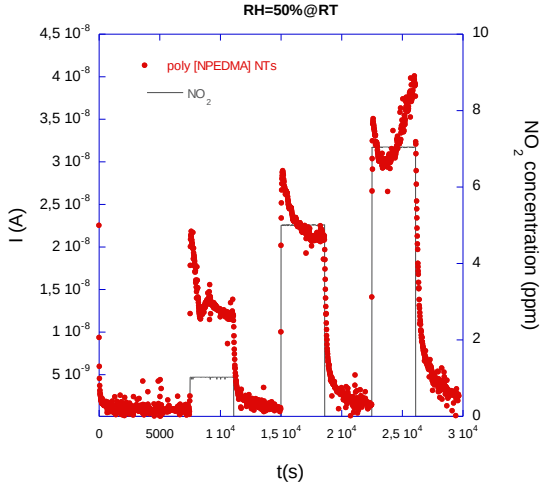

Figure 2. Current variation of PANI nanotubes exposed to NO_2.

NUOVO SENSORE PER LA MISURA DELL'H_2O_2 BASATO SU ELETTRODI STAMPATI MODIFICATI CON CARBON BLACK

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I materiali nanostrutturati, in particolare i nanotubi di carbonio a parete singola e multipla, stanno attualmente riscuotendo grande interesse, vista l'importanza esponenziale che stanno acquisendo le nanotecnologie e vista la possibilità, in campo elettrochimico, di determinare con tali materiali alcune molecole con elevata sensibilità.
Negli ultimi anni il nostro gruppo di ricerca ha sviluppato e caratterizzato elettrodi modificati con carbon black (CB), materiale nanostrutturato a basso costo usato principalmente come stabilizzante per la protezione dai raggi ultravioletti (UV) in applicazioni plastiche e nella produzione di pneumatici. Tali sensori sono stati testati mediante voltammetria ciclica (CV) per diverse molecole quali NADH, epinefrina, norepinefrina, benzochinone, dopamina, mostrando un aumento della corrente di ossidazione, e in alcuni casi, quali epinefrina, norepinefrina e benzochinone una diminuzione del ΔE_p [1].
In questo lavoro di ricerca sono stati utilizzati elettrodi stampati presso il nostro laboratorio e successivamente modificati con materiale nano strutturato. I sensori così ottenuti sono stati caratterizzati elettrochimicamente tramite voltammetria ciclica, e morfologicamente mediante microscopio a scansione elettronica.
La modifica degli elettrodi stampati tramite deposizione di una dispersione di CB in DMF/H_2O 1:1 (v/v) ha rivelato, utilizzando il ferricianuro come molecola-modello, le migliori prestazioni elettroanalitiche, grazie probabilmente alla maggiore quantità di CB presente sull'elettrodo di lavoro, come evidenziato dallo studio morfologico.
Gli esperimenti effettuati per valutarne la stabilità operativa e a lungo termine hanno dimostrato che il sensore risulta stabile sia dopo 50 CV che dopo 3 mesi, mantenuto a temperatura ambiente.
Studi in CV utilizzando l'acqua ossigenata (H_2O_2) hanno evidenziato un aumento della corrente di ossidazione e una diminuzione del potenziale di ossidazione. Tali risultati mostrano per la prima volta l'effetto elettrocatalitico del CB anche verso il perossido di idrogeno. Gli elettrodi modificati con CB sono stati poi utilizzati per la misura amperometrica dell'H_2O_2, ottenendo un intervallo di linearità compreso tra 5×10^{-6} e 1×10^{-3} M, un basso limite di rilevabilità, pari a 5×10^{-6} M (rapporto segnale/rumore=3) ed una sensibilità uguale a 203 mA M^{-1} cm^{-2}, dimostrando che possono essere utilizzati sia come sensori che come trasduttori per lo sviluppo di biosensori che utilizzano enzimi ossidasi.

Riferimenti

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SVILUPPO DI UN BIOSENSORE ELETTROCHIMICO PER LA MISURA DI PESTICIDI ORGANOFOSFORICI TRAMITE SELF ASSEMBLED MONOLAYER DI CISTEAMMINA E ACETICOLINESTERASI

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In questo lavoro di ricerca è stato sviluppato un biosensore amperometrico per la misura di pesticidi basato sull'inibizione dell'enzima acetilcolinesterasi immobilizzata su elettrodi stampati d'oro mediante cisteammina e glutaraldeide. L'attività enzimatica è stata monitorata misurando l'ossidazione del prodotto enzimatico, la tiocolina, mediante l'utilizzo in soluzione del mediatore elettrochimico ferricianuro, ad un potenziale applicato pari a +400 mV vs Ag/AgCl.

E' stata dapprima valutata l'attività elettrocatalitica del ferricianuro sulla reazione di ossidazione della tiocolina; in seguito l'elettrodo stampato d'oro è stato testato per la misura della tiocolina in amperometria utilizzando il ferricianuro in soluzione, ottenendo un intervallo di linearità compreso tra 3×10^{-6} M e 5×10^{-5} M con un limite di rilevabilità di 1×10^{-6} M (S/N=3).

E' stato poi valutato l'effetto del grado di ricopertura della superficie dell'elettrodo di lavoro d'oro da parte del tiolo cisteammina sulla reazione di ossidazione del ferrocianuro, prodotto dalla reazione tra tiocolina e ferricianuro con l'ausilio del disegno sperimentale. L'elettrodo stampato modificato con il layer di cisteammina è stato quindi utilizzato per immobilizzare l'enzima acetilcolinesterasi mediante l'ausilio della glutaraldeide.

Tale biosensore è stato testato utilizzando il paraoxon come pesticida modello, ottenendo un intervallo di linearità compreso tra 2 e 40 ppb ed un limite di rivelabilità di 2 ppb corrispondente al 10% di inibizione. Campioni reali di acqua di fiume e di rubinetto sono inoltre stati analizzati ottenendo risultati soddisfacenti in termini di recupero e riproducibilità.

DETERMINAZIONE DELL'ALFA-AMILASI MEDIANTE SISTEMA BIENZIMATICO IN FLUSSO

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L'α-amilasi è una delle principali componenti proteiche presenti nella saliva umana. Quest'enzima salivare è il principale responsabile del processo iniziale di digestione dei carboidrati [1], oltre ad essere importante per l'immunità delle mucose del cavo orale, in quanto inibisce l'adesione e la crescita dei batteri sui denti. Negli ultimi anni si ritiene che la misura di questo enzima sia uno strumento utile come indicatore di diverse patologie, tra cui l'obesità, la carie e quelle legate allo stress.

In questo lavoro viene proposto un metodo non invasivo per la determinazione dell'α-amilasi in saliva, dato il crescente interesse per questo analita. Il metodo si avvale di una catena di trasformazioni enzimatiche dei prodotti di idrolisi dell'α-amilasi e di un sistema bienzimatico con trasduzione amperometrica, costituito da un biosensore a glucosio (immobilizzazione dell'enzima *glucosio ossidasi* su un elettrodo stampato modificato con Prussian Blu) in linea con un reattore a membrana (immobilizzazione dell'enzima *α-glucosidasi*) posto in un sistema in flusso (*Flow Injection Analysis* FIA). La determinazione dell'*α-amilasi* avviene quindi indirettamente mediante la misura dei suoi prodotti di idrolisi che innescano una catena di reazioni enzimatiche (formazione di glucosio dai prodotti di idrolisi dell'α-amilasi ad opera dell'*α-glucosidasi* e di H_2O_2 dal glucosio per l'azione della *glucosio ossidasi*), il cui prodotto finale (H_2O_2) è determinato amperometricamente. Il sistema è dotato di due canali, il primo collegato al biosensore a glucosio, l'altro che attraversa il reattore a membrana per poi arrivare al biosensore stesso. Il motivo è da associare alla presenza di glucosio endogeno nella matrice saliva che si andrebbe ad addizionare a quello prodotto dalla catena di reazioni enzimatiche utilizzate per la determinazione dell'*α-amilasi*. In questo modo la concentrazione che si otterrà per questo enzima sarà data da una misura differenziale, direttamente proporzionale alla sola concentrazione di *α-amilasi* presente.

Per tale sistema sono stati ottimizzati tutti i parametri operativi, quali il tempo di reazione per ciascuna reazione enzimatica, la concentrazione del substrato, la velocità di flusso e l'effetto della matrice sull'analisi. Le misure in matrice non hanno evidenziato la presenza di interferenti di tipo elettrochimico o enzimatico, e le misure sono state eseguite con una semplice diluizione della saliva 1:1 (v/v) in tampone di lavoro prima dell'analisi. La riproducibilità delle misure in saliva (n=6) è dell'ordine del 6%, mentre il recupero effettuato addizionando soluzioni standard di enzima a campioni di saliva "bianca" è risultato intorno al 95%. Il metodo proposto ha mostrato in matrice un intervallo di linearità compreso tra 10 – 500 $IUmL^{-1}$ con un limite di rilevabilità pari a 9 $IUmL^{-1}$ per l'enzima in esame. Il tempo richiesto per l'analisi completa di un campione è risultato essere di 35 min.

Sono stati anche analizzati dei campioni reali di saliva prelevati da volontari a digiuno il mattino appena alzati e, per alcuni di questi campioni, i risultati sono stati confrontati con quelli ottenuti dall'applicazione di un kit commerciale spettofotometrico (Salimetrics LLC) [2]. I risultati sono stati soddisfacenti, con un errore relativo intorno al 7%, quindi i due metodi si mostrano in buon accordo.

Riferimenti

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MODELLO DI UN SAGGIO PSEUDO-OMOGENEO BASATO SU MAGNETO-IMMUNOSENSORI ELETTROCHIMICI

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In questo lavoro viene presentato un sistema modello finalizzato allo sviluppo di un saggio pseudo-omogeneo, basato su magneto-immunosensori elettrochimici, per la rivelazione di tossine botuliniche.
Particelle magnetiche (IMBs) sono state utilizzate come supporto per l'immobilizzazione di IgG di rabbit, capaci di interagire selettivamente con anti-IgG coniugate con HRP, enzima utilizzato come marcatore e per il quale sono state testate elettrochimicamente due differenti coppie di substrati: idrochinone + H_2O_2 e ioduro di potassio + H_2O_2. Tale studio ha previsto sia l'uso di elettrodi screen-printed (SPEs) pristine che modificati con Prussian Blue (PB), mediatore elettrochimico molto selettivo per la misura dell'H_2O_2. Impiegando come tecnica di rivelazione la cronoamperometria, sono state effettuate misure di attività enzimatica: dal confronto dei risultati ottenuti con entrambi i tipi di sensore, l'idrochinone è risultato essere il substrato più appropriato per la misura dell'attività dell'HRP (intervallo di linearità 0.01-0.1 U/mL), e gli SPEs modificati con PB, grazie al basso potenziale applicato (-50 mV), sono stati selezionati come trasduttori elettrochimici. Successivamente, l'enzima glucosio ossidasi (GOD) è stato immobilizzato (con una riproducibilità dell'11%) sulla superficie di strisce a 8 SPEs-PB.
Il saggio proposto prevede il trasferimento di un'appropriata quantità di IMBs (con rabbit IgG) in una serie di piccole 'reservoir' contenenti tampone fosfato, idrochinone e anti-IgG-HRP (a varie diluizioni da analizzare). Dopo 30 minuti di incubazione, l'intero contenuto di ogni 'reservoir' viene trasferito nei pozzetti della strip e le IMBs vengono concentrate, con l'ausilio di un apposito supporto magnetico, sulla superficie degli SPEs-PB-GOD (Fig. 1). La corrente di fondo viene registrata in cronoamperometria per 30 secondi, al termine dei quali viene addizionato glucosio ai pozzetti e monitorata la variazione di corrente per 60 secondi.
Un interessante aspetto di questo dispositivo è la non necessità di eseguire lavaggi intermedi dato che l'H_2O_2, prodotta dalla GOD sulla superficie del sensore, reagisce preferenzialmente con l'HRP dell'immunocomplesso legato alla fase solida e non con gli anticorpi-HRP rimasti liberi in soluzione.
Tale sistema rappresenta un modello per lo sviluppo di un saggio pseudo-omogeneo mirato alla determinazione di tossine botuliniche o di altri target ad alto peso molecolare. In questo caso anticorpi monoclonali, capaci di intergire selettivamente con l'analita target, verranno immobilizzati sulla superficie delle particelle magnetiche e, poiché il format previsto è di tipo 'sandwich', sarà richiesto anche l'uso di anticorpi policlonali coniugati con l'enzima HRP.

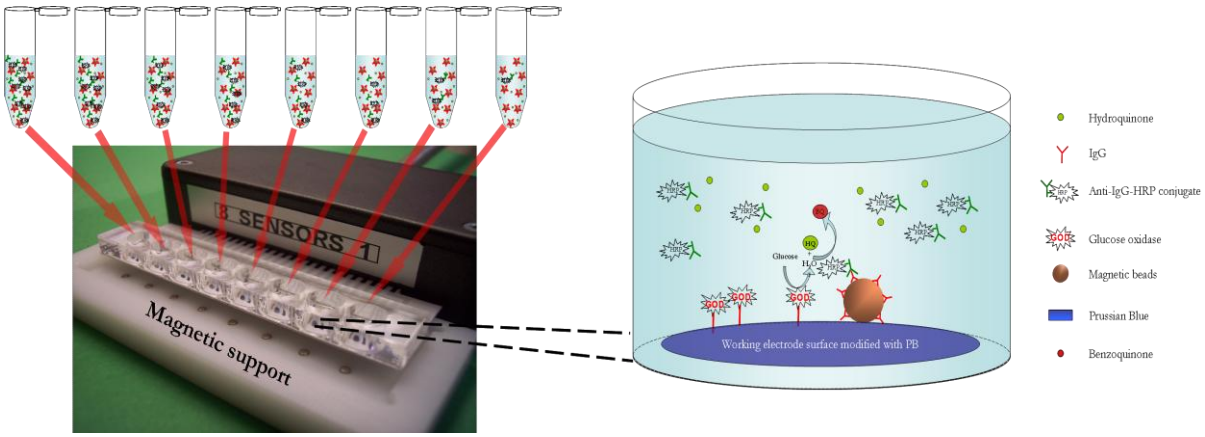

Fig. 1 Schema del saggio pseudo-omogeneo elettrochimico

Gli autori desiderano ringraziare il progetto PRIN 2007 per il finanziamento ricevuto

COLLISIONAL MECHANISM BASED E-DNA SENSORS: A GENERAL PLATFORM FOR LABEL-FREE ELECTROCHEMICAL DETECTION OF DNA BINDING PROTEINS AND PROTEIN/SMALL MOLECULE INTERACTIONS

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Here we demonstrate a reagentless, electrochemical platform for the specific detection of proteins that bind to single- or double-stranded DNA. The sensor is comprised of a double- or single-stranded, redox-tagged DNA probe which is covalently attached to an interrogating electrode. Upon protein binding the current arising from the redox tag is suppressed, indicating the presence of the target. Using this approach we have fabricated sensors against the double-stranded DNA binding proteins TATA-box binding protein and M.HhaI methyltransferase, and against the single-strand binding proteins E. coli SSBP and replication protein A. All four targets are detected at nanomolar concentrations, in minutes, and in a convenient, general, readily reusable, electrochemical format. The approach is specific; we observed no significant cross-reactivity between the sensors. Likewise the approach is selective; it supports, for example, the detection of single strand binding protein directly in crude nuclear extracts.

The generality of our approach (including its ability to detect both double- and single-strand binding proteins) supports a collisional signaling mechanism in which binding alters the collision efficiency, and thus electron transfer efficiency, of the attached redox tag.

We have demonstrated this hypothesis designing a general, sensitive and selective approach for the detection of proteins binding to specific small molecules. The electrochemical approach is based on a redox-tagged DNA signaling scaffold conjugated to a small molecule receptor and covalently attached to an interrogating electrode. In the absence of target, this scaffold is relatively dynamic, allowing collisions between the redox tag and electrode surface and supporting relatively efficient electron transfer. The binding of a protein to the small molecule receptor alters the dynamics of the scaffold, minimizing collisions and thus reducing the observed current. We optimized the scaffold using a biotin receptor to determine optimal structure before then applying it to detection of anti-digoxigenin antibodies using the steroid drug as the receptor. Both sensors are sensitive (detection limits in the low nanomolar), rapid and selective enough to function directly in complex matrices, including blood serum, soil suspensions and foodstuffs.

DETERMINAZIONE DELLA PALITOSSINA MEDIANTE UN SENSORE ELETTROCHIMICO ACCOPPIATO AD UN SAGGIO EMOLITICO

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La palitossina (PLT) è uno dei più potenti composti naturali non proteici conosciuto fino ad oggi, esibendo un'estrema tossicità nei mammiferi ($D.L._{50}$ 10-100 ng/Kg). Tale tossina è prodotta da alghe appartenenti al genere *Ostreopsis,* che in questi ultimi anni ha colonizzato i mari italiani provocando fenomeni di intossicazione a carico dell'apparato respiratorio in individui esposti ad aerosols. E' stata riscontrata in diversi organismi marini rappresentando così un rischio per la salute umana. La PLT legandosi alla pompa Na^+/K^+-ATPasi determina uno squilibrio ionico con conseguente lisi cellulare.

Scopo di questo lavoro è lo sviluppo di un sensore elettrochimico accoppiato ad un saggio emolitico per la determinazione della palitossina e dei suoi congeneri. Il test si basa sulla misura amperometrica dell'attività della lattico deidrogenasi (LDH) rilasciata nel mezzo quando eritrociti di montone, cellule particolarmente ricche di tale enzima (e che non necessitano di semina, crescita e mantenimento in opportuni terreni colturali), vengono lisati a seguito dell'incubazione con PLT e con i suoi congeneri. L'entità dell'emolisi, e quindi la quantità di LDH misurata, è correlabile alla concentrazione delle tossine in oggetto.

La reazione catalizzata dall'enzima LDH è la seguente: piruvato + NADH→ lattato + NAD^+.

Dopo un breve periodo di incubazione enzima-subsrato, il NADH residuo (la cui quantità dipende dalla concentrazione di LDH nel mezzo) può essere misurato elettrochimicamente utilizzando opportuni mediatori secondo le reazioni sequenziali:

NADH + PMS →NAD^+ + PMSH;

PMSH + 2 $Fe(CN)_6^{3-}$→ PMS + 2 $Fe(CN)_4^{2-}$+ H^+ +2e^-

La riossidazione del $Fe(CN)_4^{2-}$ alla superficie di un elettrodo screen-printed (SPE) genera un segnale di corrente inversamente proporzionale alla concentrazione di LDH.

Uno studio di fattibilità ha riguardato la scelta del potenziale di lavoro (+260 mV), l'ottimizzazione della concentrazione di PMS e $Fe(CN)_6^{3-}$ e una misura di attività enzimatica che ha mostrato (dopo un periodo di incubazione di 15 min a T.A.) un intervallo di linearità tra 0.025 e 0.2 U/mL di LDH. Si è proceduto alla messa a punto del protocollo di emolisi che ha previsto la preliminare ottimizzazione della diluizione di sangue da utilizzare (1:20), la valutazione di eventuali interferenze elettrochimiche e la successiva incubazione (24 h a 25°C, in piastra da 96 pozzetti) di piccoli volumi di sangue (1:20) con uguali volumi di soluzioni standard di PLT. A seguito dell'aggiunta di piruvato + NADH (lasciati incubare per 15 min) e poi di PMS + $Fe(CN)_6^{3-}$(incubati per 1 min), il contenuto di ogni pozzetto è stato trasferito su strisce da 8-SPEs ed è stato possibile misurare concentrazioni di PLT dell'ordine dei ppt. Si è inoltre dimostrato che la preincubazione degli eritrociti con oabaina preveniva la risposta emolitica indotta dalla palitossina. Sono in corso prove per abbreviare la durata del saggio emolitico, a cui seguiranno test di selettività e l'analisi di campioni sperimentalmente e naturalmente contaminati.

SVILUPPO DI UN GENOSENSORE PER LA RICERCA DI *LISTERIA MONOCYTOGENES*

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Nel presente lavoro viene descritto lo sviluppo di un genosensore per la ricerca di *Listeria monocytogenes* basato su una sonda a DNA per il gene dell'internalina A (inlA) (1) utilizzata per la modifica di elettrodi stampati a base d'oro (AuScreen Printed Electrodes - AuSPEs) (3).
Il genosensore è un biosensore elettrochimico a DNA, che si basa sul riconoscimento di un singolo filamento di DNA (ssDNA) mediante la "misura" dell'ibridazione con un filamento oligonucleotidico complementare.
Il protocollo di misura della ibridazione della sonda per la ricerca del gene dell'internalina A in DNA di *L. monocytogenes* è stato sviluppato a partire dal filamento oligonucleotidico complementare e quindi applicato su campioni reali di DNA di Listeria.
Il sensore è stato disegnato immobilizzando la sonda di DNA, attraverso legame covalente delle amine libere delle basi del DNA, alla superficie degli elettrodi d'oro stampati (Au-Screen Printed Electrodes) modificati con membrana (carboxylate terminated alkanethiol) auto-assemblata SAM (self-assembled monolayers) e mediante l'attivazione con N-idrossi-sulfo-succinimide (NHS) e N-(3-dimetilamino)propyl-N-etilcarbodiimide idrocloruro (EDC) (2, 3).
La sonda immobilizzata era in grado di ibridare il suo complementare. L'avvenuta ibridazione veniva rilevata grazie all'utilizzo di Blu di Metilene come marcatore elettroattivo dell'ibridazione.
Come tecnica per la rilevazione elettrochimica del segnale è stata utilizzata la *Differential pulse voltammetry (DPV*). Sono state studiate le variazioni di intensità del picco di corrente per la sonda non ibridata DNA e per la sonda ibridata con il suo oligonucleotide di sintesi complementare. Il sistema è stato quindi applicato a DNA di *L. monocytogenes* e Listerie non monocytogenes.

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Progetto 1235/2009.0710 cofinanziato dalla Fondazione Banco di Sardegna.

FUNCTIONALIZED GRAPHENE NANORIBBONS FROM THE OXIDATIVE UNZIPPING OF SWCNTS AND MWCNTS FOR SENSOR APPLICATIONS.

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The functionalized few layers of graphene nanoribbons were successfully synthesized by oxidative unzipping of single wall carbon nanotubes (SWCNTs) [1]. The nanoribbons produced from SWCNT were fully characterized by the FT-IR, Raman, X ray photoelectron (XPS) spectroscopy and Thermal analysis (TGA/DTA). For the topographic study of the product obtained from the SWCNT unzipping reaction, the transmission electron microscopy (TEM), atomic force microscopy (AFM) and scanning tunnelling microscopy and spectroscopy (STM/STS) were used, highlighting the typical graphene nanoribbons structure [1]. In particular, the STM/STS study was very useful to characterize not only the topographic features of these few-layers of graphene-FLG but also the electronic properties of graphene nanoribbons (especially after the functionalization induced by the oxidation and reduction treatments). In addition, a comparative study with the oxidized and reduced (in particular, this last, produced following a specific treatment with hydrazine in solution) graphene nanoribbons, obtained from the unzipping of multi wall carbon nanotubes (MWCNTs), has been also performed. The same characterization study (i.e.; the morphological and structural investigations) was also carried out for the oxidized and reduced graphene nanoribbons from MWCNTs [2]. Finally, interesting analytical applications have been proposed by the assembling of a glucose biosensor that could be useful to detect glucose in biomedical applications, and a FET device based on the electronic properties of the functionalized graphene nanoribbons.

Figura 1. The single layer of oxidized graphene nanoribbons from the oxidative unzipping of the SWCNTs, as precursor.

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IMPEDANCE CHARACTERIZATION OF LIPID LAYERS EMPLOYED IN ORGANIC THIN FILM TRANSISTOR FOR BIOSENSING APPLICATION

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Bio and chemical sensing represents one of the most attractive application of organic electronics. Organic Thin Film Transistor (OTFT) biosensors are in this respect, very promising and have shown the potential to offer very high performance level[1]. Organic electronics allows to fabricate sensing circuits, also in an array configuration[2], on flexible, plastic or even paper substrates, by low cost printing compatible procedures. This can open interesting perspectives for the development of paper test-strip that combine low cost, reliability with label-free electronic detection and data processing.

The use of cell-membrane mimics such as liposome and lipid bilayers has recently attracted great attention as biological recognition layer in OTFT biosensors and several immobilization methods on a solid support have been reported[3]. Supported membranes show an intrinsically low bioactivity, making them interesting as an interface between the non-biological material on the surface of OTFT and biologically active fluids.

To investigate the dielectric properties of supported lipid bilayer membrane (sBLM) such as electrical resistance and capacitance, impedance spectroscopy has been employed. In particular, two class of experiments were performed. In the first one, sBLM has been characterized directly on a metal support and in the second an organic semiconductor was used to improve the quality of the supported lipid bilayer. The sBLMs structure in the two systems is explained in terms of equivalent circuits composed of simple electrical elements such as resistors and capacitors. Information on the presence of lipid bilayer inhomogeneities are gathered from the electrical parameters determined by fitting the frequency-dependent impedance of the equivalent circuits to the measured data. Furthermore, X-ray photoelectron spectroscopy was employed for the characterization of the active layers, in terms of surfaces and interfaces quality, e.g. surface elemental composition, chemical speciation, in-plane homogeneity, etc..

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VOLTAMMETRIC DETERMINATION OF DISPERSE RED 13 DYE IN SUPERFICIAL WATER BY POLY-L-GLUTAMIC ACID MODIFIED ELECTRODE

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Disperse azo dyes are widely used in a variety of products, such as textile industry for dyeing synthetic fabrics such as polyester, nylon, acetate, cellulose, acrylic, etc[1]. The disperse dyes bears azo (–N=N–) groups and nitro substituents, presents low water solubility and can promote great environmental and health problems when lost during the fiber dyeing process. Great concern have been dispended to this kind of dyes in the last years, since they are catalogued as the most positive mutagenic responses among several types of dyes present in industrial effluents[2]. The detection of these dyes as contaminant in superficial water of rivers, lakes and drinking water requires specific methods, able to identify and quantify these compounds at very low concentration. The present work describes the development of modified electrode with poly-L-glutamic acid (PGA) as a polymer able to mimetize complex biological processes. The PGA is a synthetic polyaminoacid with repetitive units of glutamate, where the free carboxyl group offers strategic points to interact with amine groups present in the Disperse Red 13, used as a model compound. Voltammetric measurements were performed with an AUTOLAB PGSTAT 30 potentiostat connected to a microcomputer controlled by software GPES 4.9 for data acquisition. A three electrode system (EG&G PARC) consisting of an SCE as reference electrode, a platinum wire as auxiliary electrode and a glassy carbon electrode as working electrode were used. The poly-L-glutamic acid films modified electrode was obtained by an aliquot of 10 mL of an aqueous polymeric solution of PGA (1% w/v) placed on the polished electrode surface, which was dried at room temperature. The electrode was removed, placed in a phosphate buffer pH 6 and submitted to 10 cycles between -0.8 V to +2.0 V at 100 mV s^{-1}. Cyclic voltammograms obtained on the glassy carbon electrode presented a reduction peak at -0.61 V in the conventional electrode and two reduction peaks at -0.44 V and -0.99 V with higher peaks intensities on the modified electrode by films of PGA due nitro group reduction. After optimization of the best experimental conditions it was possible to construct calibration curves fro the Disperse Red 13 in Bu4NBF4/DMF dye determination using differential pulse voltammetry from 0.25 to 3.00 μmol L^{-1}, following the equation: I_{pc} (μA) = 0.633 + 861663.08 + C (C = μ mol L^{-1}), correlation coefficient of 0.997 for n = 10. The limit of detection [3 x (standard deviation of blank) / sensitivity] was 0.015 μmol L^{-1}. Aliquots of 1.2 mL of the wastewater sample (Araraquara, SP, Brazil) spiked to 5.00 μg L^{-1} of the dye, were transferred to a solid-phase extraction cartridge containing 1.00 g of reverse phase C-18. The resultant solution was submitted to the differential pulse voltammetric analysis using the best conditions previously optimized. The quantification of the Dispersed Red 13 dye was performed using the standard addition method with recovery of values around 100%.

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DUE NUOVI IMMUNOSENSORI A MISURA DIRETTA PER LA DETERMINAZIONE DI PROTEINE

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Con l'intento di rimpiazzare gli immunosensori "competitivi" da noi realizzati negli scorsi anni, è stato condotto uno studio di nuovi immunosensori elettrochimici diretti, sviluppati per l'analisi di alcune proteine contenute nel siero (immunoglobuline G), o nel latte (lattoferrina) [1-3]. Nelle ricerche precedenti il procedimento operativo di misura era quello classico "a competizione", nella presente ricerca invece è di tipo "diretto"; anche in questo ultimo caso tuttavia, a differenza di quanto riportato di solito in letteratura per immunosensori di questo tipo, in cui il segnale viene spesso ottenuto direttamente in seguito alla formazione dell'immuno-complesso (che ad esempio può causare la variazione del potenziale di membrana), è stato ancora una volta utilizzato un marker enzimatico, di cui ci si è serviti per eseguire la misura elettrochimica, sia che il trasduttore impiegato fosse amperometrico, oppure potenziometrico. Per poter realizzare la misura diretta su un campione reale, è stato perciò necessario sviluppare procedimenti innovativi. E' stata innanzitutto misurata sperimentalmente "una tantum" la concentrazione dell'antigene, o di anticorpo, marcato necessaria a formare il complesso coniugato con il rispettivi anticorpo, o antigene, immobilizzato su apposita membrana. La misura veniva poi effettuata facendo coniugare l'antigene da misurare, contenuto nel campione reale, con l'anticorpo (immobilizzato, od in soluzione), infine "completando" la coniugazione tra anticorpo immobilizzato ed una quantità fissata di antigene marcato, oppure tra antigene immobilizzato in membrana ed una quantità fissata di anticorpo marcato. Veniva quindi effettuata la misura enzimatica per aggiunta dell'apposito substrato. Infine si otteneva il segnale, con cui entrare nella curva di calibrazione, effettuando la differenza tra il segnale registrato e la misura "una tantum" e quello ottenuto mediante la misura sopra descritta. Per l'immunosensore per le HIgG (immunoglobuline) è stato sempre impiegato un trasduttore potenziometrico a diffusione gassosa per l'NH_3, l'enzima marker utilizzato è stato sempre l'ureasi. Mentre nel caso degli immunosensori per la lattoferrina, sono stati impiegati alternativamente due diversi tipi di trasduttori elettrochimici amperometrici: l'elettrodo per il perossido di idrogeno oppure l'elettrodo di Clark; l'enzima marker utilizzato è stato in questo caso sempre la perossidasi (HRP), la cui coniugazione con la lattoferrina è stata ottenuta mediante un procedimento di biotinilizzazione. Nel caso degli immunosensori per le HIgG, i dati analitici ottenuti sono stati diversi a seconda che si utilizzasse il metodo di misura diretto, oppure quello a competizione: infatti, utilizzando il primo metodo, il LOD è risultato dell'ordine di 10^{-8} mol L^{-1} e l'intervallo di linearità compreso tra 10^{-5}-$5x10^{-8}$ mol L^{-1}, mentre utilizzando il secondo metodo, il LOD è risultato dell'ordine di 10^{-10} mol L^{-1} e l'intervallo di linearità compreso tra circa $5x10^{-8}$-10^{-10} mol L^{-1}. Per la determinazione delle HIgG quindi i due procedimenti di misura permettono di ricoprire due intervalli di concentrazione abbastanza diversi entro i quali poter effettuare le misure. Mentre per la lattoferrina gli immunosensori diretti, o a competizione non sono risultati molto diversi dal punto di vista dei dati analitici ottenuti, il LOD è stato sempre dell'ordine di 10^{-8} mol L^{-1} mentre l'ampiezza dell'intervallo di linearità è risultata la stessa, circa 10^{-5} - $5x10^{-8}$ mol L^{-1}, quale che fosse il trasduttore impiegato ed il metodo (diretto, o a competizione) utilizzato.

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DEVELOPMENT OF DISPOSABLE ELECTROCHEMICAL IMMUNOSENSOR BY USING MAGNETIC BEADS FOR *PHAKOPSORA PACHYRHIZI* DETECTION IN THE EARLY DIAGNOSIS OF SOYBEAN RUST

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Asian soybean rust is a fungal disease caused by *Phakopsora pachyrhizi* which is a virulent pathogen that can quickly defoliate plants, reduce pod set, pod fill, seeds and, thus, quality, reducing crop yields by 10 to 80%. The disease is disseminated through spores (urediniospores) transported by the wind and spreads rapidly, causing loss of foliar area and a severe reduction in grain yield and, consequently, severe economic losses [1]. The appearance of the disease is recent in countries that now have large production of soybeans, such as Brazil and United States, but the infection cost was estimated at approximately $1.2 billion for past harvests, a large percentage of these losses resulting from inappropriate use of fungicides. The biggest problem is in the early detection of the fungus infestation because it is performed by a visual method. However, when the fungus is readily visible, the culture is already infected. The lesions can also be misinterpreted as non aggressive infections, which are normally found on the crop. The method used to control the pathogen is fungicide application, thus precise and early diagnosis minimizes spore dissemination, decreasing the costs of control and avoiding a continuous environmental contamination. Thus, farmers need an early identification method that could help them to control disease infestation. Due to their simplicity and sensitivity, biosensors could be effective tools for disease diagnosis and monitoring [2].

Immunosensors are based on the use of an antibody that reacts specifically with a substance (antigen) to be tested. Immobilization of the receptor (e.g., an antigen) on a substrate is convenient for applications of molecular bio-recognition for the detection of a target molecule (e.g., its antibody) present in solution. The specificity of antigen-antibody interactions allows the development of immunosensor devices for clinical diagnostics, environmental monitoring, etc. Immunosensors offer several advantages such as limited hands-on time, high-throughput screening, improved sensitivity, real-time analysis, possibility of quantification and label-free detection (i.e., antibodies do not need to be labelled with fluorophores, radioisotopes, colloidal gold particles or enzymes for detection of binding events or signal enhancement). Therefore, immunosensors can be successfully applied in agricultural, food, environmental, pharmaceutical chemistry and clinical applications.

An interesting immunoanalysis is using the the electrochemical meassurements technology. Electrochemical sensors have revolutionized modern analysis because of their simplicity and speed in response by the direct transduction to electronic equipment. For mass-fabrication with low cost, the use of screen printed electrodes is an alternative interesting to classical electrodes.

Based on these considerations, the aim of this work was the development of an electrochemical-based immunosensor using *Phakopsora pachyrhizi* mycelium antibodies for the early detection of Asian rust on the soybean leaf samples. It was used a graphite screen-printed electrodes and the antibodies was immobilized on magnetic beads for immunoassay. The use of beads is an attractive alternative, especially for the automation of analytical biosystems when irreversible sensing mechanisms are involved. The electrodes presented good analytical performances in terms of sensitivity and speed of the analysis.

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ONCHIP ELECTROCHEMICAL DETECTION OF DNA HYBRIDIZATION BY 2-ELECTRODES LINEAR SWEEP VOLTAMMETRY

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The implementation of a large number of diagnostic screening tests requires a paradigm change from the classical hospital-centred analysis lab, towards a decentralized point-of-care testing that is expected to foster the birth of personalized medicine in the near future. Low-cost, automatic fool-proof testing instrumentation and parallelized analyses will represent key technologies for the implementation of such epochal change. Even if the best limits of detection are currently obtained by using optical transducer techniques, electrochemical biosensors are promising for the implementation of highly integrated, programmable and massively parallelized analytical devices. The techniques used for such parallelized electrochemical assays are nowadays performed with strategies that derive directly from "classical" techniques which involve different types of electrodes which are usually bulky and made of materials hardly compatible with standard industrial fabrication processes. An example is the work from Choi et al. [1], where an array of microfabricated working electrodes is used with an external common Ag/AgCl reference electrode and a common Pt counter electrode. In a more modern approach all miniaturized electrodes necessary for the measurements are fabricated on-chip with a standard industrial process,

In this communication, we report the design, implementation and characterization of a 2-electrodes on-chip biosensor based on the selective binding of Hoechst 33258 [2'-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi(1H-benzimidazole)], an electrochemically active groove binder, to a DNA duplex. The electrochemical technique operates on the same principle as Linear Sweep Voltammetry (LSV), but the voltage is swept linearly between the two electrodes of the biochip, without a direct control of the voltage at the surface of the working electrode. The anodic currents are qualitatively comparable to a standard system (fig.1), and the biosensor has the same limit of detection of 10nM (fig.2) as a standard bulky 3-electrodes setup [1]. Such technique has proved to work in a device composed of several measuring micro-electrodes embedded in a microfluidic system (fig. 3), and fabricated with standard industrial processes, and is promising for applications that require massively parallelized biosensors.

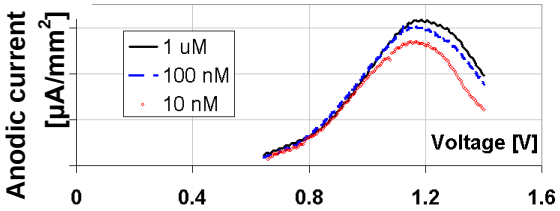

Figure 1. Typical response curves at increasing target concentration.

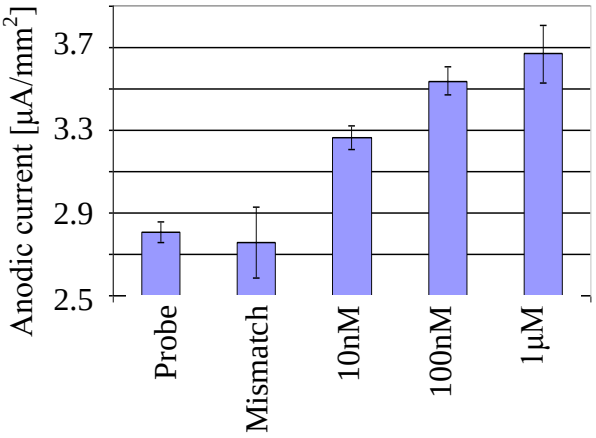

Figure 2. Calibration curve of the biosensor composed of 2-electrodes.

Figure 3. Biochip fabricated with standard industrial techniques.

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ELECTROSYNTHESIS OF MOLECULARLY IMPRINTED POLYPYRROLE FOR THE ANTIBIOTIC LEVOFLOXACIN

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Considerable scientific research is nowadays devoted to the development of tailor-made receptors capable of recognizing and binding the molecular target with high affinity and selectivity. Molecular imprinting revealed to be an attractive tool for designing selective materials. During imprinting process polymeric matrices with specific binding sites are generated by template-induced prearrangement of complementary interactive functional groups [1]. The polymerization "freezes" binding groups within the imprinted cavity. Removal of the template affords binding sites complementary in size and in chemical functionality to the original template [1]. A molecular memory is thus introduced into the polymer that is able to recognize the template and to selectively interact with it during the rebinding step [1]. Among different analytical applications for which Molecularly Imprinted Polymers (MIPs) have been successfully used [2], MIP-based sensors attract increasing attention on account of their specificity, stability, robustness and low cost [3]. A particularly important aspect in the design of MIPs sensors is MIP integration with the transducer and different strategies have been employed to address this key issue. Among these, MIP electropolymerization has emerged as an effective way of synthesizing and anchoring MIP to the transducer surface [4]. By this approach the film grows on the electrode surface, so good adherence is guaranteed. Moreover, film preparation is simple and quick and its thickness is controlled by varying the amount of circulated charge. During the last decade, several electrosynthesized MIPs have been proposed as recognition element, particularly in electrochemical sensors [see e.g. 5-6].
The present work presents preliminary results about the development of an electrosynthesized imprinted polypyrrole (PPY) to be used for the selective detection of a fluoroquinolone antibiotic (levofloxacin). Experimental conditions for the electropolymerization of PPY in the presence of the template were optimized. The as prepared MIP has been characterized by X-Ray Photoelectron Spectroscopy (XPS) to verify the template entrapment in the polymeric matrix. Different washing protocols have been tested by UV-vis and HPLC analysis of the washing solutions. After the selected washing treatment, imprinted films were analyzed by XPS and a very satisfactory template removal was estimated being equal to 85%. Rebinding experiments were performed by exposing the imprinted PPY to levofloxacin solutions, subsequently analyzed by HPLC. The effect of solvent and of time exposure has been thus investigated. A remarkable imprinting effect was verified by comparing rebinding performances of MIP and NIP (a blank polymer, prepared in the same conditions but in the absence of the template). Also selectivity tests have been carried out with the same experimental scheme and results will be described.

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PREPARATION AND CHARACTERIZATION OF COPPER NANOPARTICLES/POLY-3-METHYLTHIOPHENE COMPOSITE AND ITS APPLICATION TO GLUCOSE SENSING

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Nanostructured materials including nanoparticles, nanowires and nanotubes have been intensively investigated due to their size effect and to their unique chemical and physical properties (e.g. enhanced mass transport, high surface area, improved signal-to-noise ratio) [1]. Nanocomposites can be fabricated not only with different nanostructured materials, but also with various conducting polymers to possess unique hybrid properties characteristic of neither the incorporated components nor the host matrices [2]. Different approaches have been reported in literature for the entrapment of metal nanoparticles in conducting polymers [see: e.g. 3-5] and their catalytic properties have been investigated [6-8].
The present work describes the preparation and the characterization of an electrosynthesized film of poly-3-methylthiophene modified by copper nanoparticles (P-3MT/CuNPs). The deposition of copper was achieved by applying a potential pulse program [9] both on Pt and on screen-printed electrodes. The microscopic characterization of the deposit was performed by scanning electron microscopy/energy dispersive X-ray analysis (SEM) and showed a correlation between the pulse width and the amount and size of the deposited particles. The nanocomposite P-3MT/CuNPs was analyzed also by X-ray photoelectron spectroscopy (XPS). The electrocatalytic properties of P-3MT/CuNPs towards glucose oxidation were investigated and the composite film deposited on SPE was used for glucose detection in a flow-injection analysis system. The effect of the applied potential as well as of the flow rate of carrier stream was evaluated: under the selected experimental conditions, the sensor revealed a satisfactory response in terms of detection limit, linear range and repeatability.

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A NEW POTENTIOMETRIC UREA BIOSENSOR BASED ON UREASE IMMOBILIZED IN A ELECTROSYNTHESIZED POLY(*o*-PHENYLENEDIAMINE) FILM

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The urea determination is of great interest in different fields as pharmaceutical and food industry, environmental protection, fertilizers, but the most important applications are in biomedical and clinical analysis [1-3].Several urease biosensors based on modified electrodes have been reported in literature [4-5]. In this communication we propose a simple and novel potentiometric biosensor for urea detection. It was for the first time prepared by employing an electrosynthesized polymer with buffering capacity. The modified electrode was obtained by immobilization of urease in a poly-*o*-phenylenediamine (PPD) matrix grown by cyclic voltammetry in the range -0.1-+1.0 V (scan rate 100 mV/s) vs. Pt (QRE) on a glassy carbon (GC) electrode, using an unconventional "upside-down" (UD) geometry [6-7]. The effective enzyme immobilization in PPD film was verified by UV-vis exsperiments. The response of Ur/PPD film resulted linearly related to urea concentration in the range 10 μM – 1 mM with a slope of 15 mV/mM (R^2 = 0.9999). The sensor exhibits a sufficient sensitivity up to 20 mM for practical determinations, rapid response and long term stability.
Are projected study for surface characterization of the film with bulk analysis by spectroscopy techniques (e.g. FT-IR and XPS spectroscopy) aiming to a more exhaustive study of chemical modifications of film after interaction with urea and, thus to a deeper comprehension of mechanism involved in the chemical process.

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A NEW FREE ENZYMATIC SENSOR BASED ON PLATINUM–TELLURIUM MICROMATERIALS

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The control of the shape and the orientation of nano-microcrystallites as well as the ability to order and align them onto various types of substrates represent essential tasks to fulfill the creation of novel smart and functional thin film materials [1]. Since the discovery of multi- and single-walled carbon nanotubes [2], tubular structures have received considerable attention because of their potential uses in guest encapsulation, nano- or micro-electronic.

Analytical determination of glucose and hydrogen peroxide represents two important topic of environmental, pharmaceutical, clinical and industrial interest as reported in most recent works [3-4]. In such context several electrochemical sensor and biosensors modified utilizing metal, carbon nano-tubes, transition metal oxides and conducting polymers modified electrodes, have been studied analysing their response to glucose and hydrogen peroxide [5-6]. However, to the best of our knowledge, there are no reports about the detection of this two target using Te modified electrodes. In the present work an original and easy amperometric free enzymatic sensor based on a Pt electrode modified with Te microtubes was proposed. Te microtubes were synthesized by thermal evaporation of Te powder and showed a tubular structure with a hexagonal cross-section and are open ended [7]. The modified electrode was prepared by drop casting on a platinum electrode surface a mixture of Te microtubes in ethanol.

The spectroscopic characterization of Te microtubes as synthesised and Pt-Te-microtubes modified electrodes, was performed by SEM, EDX and XPS analysis. Electrochemical characterization showed that the Pt–Te microtubes modified material gives good and sensitive amperometric response to respect both glucose and hydrogen peroxide detection.

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SWCNT-CME PER LA DETERMINAZIONE DI ANTIPSICOTICI ATIPICI

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Sono presentati i risultati ottenuti con un metodo voltammetrico per la determinazione di due psicofarmaci, olanzapina e risperidone, tra i più usati ma fino ad ora poco studiati dal punto di vista elettrochimico. In particolare sono stati utilizzati elettrodi chimicamente modificati (CME) di nuova generazione, preparati con nanotubi al carbonio a parete singola (SWCNT), che grazie alle loro proprietà elettrocatalitiche consentono di poter fare determinazioni elettrochimiche in intervalli di potenziale inaccessibili agli elettrodi solidi convenzionali.
Sono stati valutati i parametri caratteristici del CME e i parametri elettrochimici necessari per caratterizzare il processo redox: controllo della diffusione o dell'adsorbimento, numero di elettroni e protoni coinvolti, coefficiente di diffusione.
L'ossidazione dell'olanzapina è caratterizzata da un processo bielettronico, con il coinvolgimento di un protone. Nel caso del risperidone l'ossidazione è caratterizzata da un processo monoelettronico, con il coinvolgimento di un solo protone.
Gli eccipienti presenti nei formulati farmaceutici non interferiscono nella determinazione. Altrettanto vale per la fluoxetina, farmaco spesso associato all'olanzapina.
I dati ottenuti con le tecniche voltammetriche sono stati implementati con informazioni ottenute tramite metodi computazionali e HPLC-MS previa coulombometria esaustiva.
Si sono quindi messe a punto le condizioni sperimentali per la determinazione di questi principi attivi in preparati farmaceutici di uso commerciale, ottenendo per l'olanzapina un limite di rivelabilità di 0.3 mg L^{-1} e per il risperidone di 0.4 mg L^{-1}.

CHEMILUMINESCENCE LATERAL-FLOW IMMUNOASSAY FOR FUMONISIN USING "CONTACT" IMAGING DETECTION

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Mycotoxins are toxic secondary fungal metabolites with deleterious effects for humans and animals. Due to the widespread occurrence of fungal contamination in foodstuff and feeds, many efforts have been made towards the development of screening methods for sensitive and rapid detection in a non-laboratory environment, mainly based on immunochemical methods.
The growing interest in point-of-use applications in the food industry has prompted the development of alternative immunoassay formats, such as lateral-flow immunoassays (LFIA), characterized by self-testing performance thanks to high simplicity and speed of operation. However, most LFIA, which are based on the use of colloidal gold or latex particles as labels and on colour-formation due to label accumulation in definite strip zones, only allow the qualitative or semi-quantitative determination of mycotoxins, with low sensitivity of the assay[1] not suitable to fulfil the regulatory requirements.
In this work, we propose a new LFIA with chemiluminescence detection for fumonisin B1 employing horseradish peroxidase as tracer, revealed by means of a chemiluminescent substrate. Detection of the signal was performed by exploiting "contact" imaging detection, in which the analytical signal is produced directly on the surface of an imaging light sensor able to localize and quantify the signal[2].
The method, which allowed detecting fumonisin B1 down to 20 ng mL^{-1} (ppb) in 30 minutes, showed the possibility to obtain semi-quantitative detection of fumonisin B1 traces with a simple, rapid, low-cost and on-field applicable assay. The method was also tested on real corn samples, after a simple and on-field applicable extraction procedure. Results were found in good agreement with those obtained with chromatographic analysis.

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IMPLEMENTATION OF GRAVITATIONAL FIELD-FLOW FRACTIONATION AS A PRE-ANALYTICAL MODULE FOR POINT-OF-CARE TESTING DEVICES

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The "Point-Of-Care" (POC) testing approach is based on the development of portable analytical platforms suitable to perform analysis directly in any required place. To meet analytical and diagnostic requirements, a POC device should combine portability, minimum sample pre-treatment, and the possibility to perform highly sensitive simultaneous detection of several biomarkers (multiplexing) in a short assay time. POC devices should also comprise an integrated module for on-line sample pre-analytical treatment and/or clean-up to achieve high sensitivity and specificity even in complex matrices.

With this respect, field-flow fractionation (FFF), a family of flow-assisted separation techniques which can separate analytes based on their morphologic characteristics (size, shape and superficial properties) can be exploited to develop pre-analytical modules for cells or macromolecules (e.g., proteins, protein complexes or adducts) fractionation. In particular, gravitational FFF (GrFFF), exploiting the Earth gravitational field to structure the separation, appears to be particularly suited for integration in POC devices, thanks to the simplicity of its separative device, amenable to miniaturization[1].

In this work, we propose the implementation of GrFFF as a pre-analytical module of a POC device, thus providing a selectively enriched fraction for the analysis with an increase of overall analytical output. We have recently demonstrated the potential analytical applications of GrFFF coupled with chemiluminescence detection[2] and the possibility to increase its selectivity by derivatizing the fractionation device walls with biospecific reagents[3].

As a first approach, in this work we present the use of GrFFF to prepare whole blood samples for the automatic on-line analysis of alkaline phosphatase activity in serum, as a biomarker of obstructive liver diseases and bone disorders. Serum alkaline phosphatase is commonly assayed after a preliminary blood sample centrifugation to obtain serum, by employing spectrophotometric techniques. In this work, the GrFFF device is employed to separate plasma from cells components which elute with different retention times. After separation, plasma is directly addressed by means of a microfluidic system and valves to the analytical module, where a chemiluminescent substrate is added and the enzyme activity is measured by means of an on-line flow-through luminometer. The diagnostic test gave quantitative results with low sample (50 µL) and reagents consumption, short analysis time (10 minutes) and high reproducibility.

In addition, to obtain compact GrFFF devices for POC, we studied the influence of an original curvilinear geometry and miniaturization of the channel on fluidic conditions and fractionation parameters. These new channel designs resulted particularly adapt to the development of compact GrFFF tools to be easily integrated in more complex and specific analytical systems with maintenance of separative properties such as soft fractionation mechanism, simplicity, high sample recovery and selectivity.

The final aim is the development of a miniaturized GrFFF-assisted module to be integrated within a lab-on-a-chip microfluidics-based device. Such module will fractionate the sample components and then transfer them to different analytical modules where analytes are quantified by exploiting biospecific recognition reactions combined with ultrasensitive bioluminescence and chemiluminescence detection techniques.

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ELECTROCHEMICAL MULTIANALYTE DETECTION OF TUMOUR BIOMARKERS

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The goal of this work was to design a multianalyte assay with electrochemical transduction for the detection of tumour biomarkers, in complex matrices, using a simple target capturing step by antibody/nucleic acid-functionalised magnetic beads and an array of electrodes for the detection.
The chosen biomarkers were the human epidermal growth factor receptor 2 (HER2), prostate specific antigen (PSA) and a microRNA (miRNA) which can be used as diagnostic, prognostic or theranostic tools for different types of cancer.
The assay for HER2 and PSA is based on a sandwich format in which a primary monoclonal antibody anti-marker is coupled to protein A modified magnetic beads. The modified beads are then used to capture the protein from the sample solution and the sandwich assay is performed by adding a secondary monoclonal antibody anti-marker labelled with biotin. The enzyme alkaline phosphatase (AP) conjugated with streptavidin is then incubated with the complex on the beads. For the miRNA, streptavidin-coated magnetic beads are modified with a DNA probe (capturing probe) specific for the chosen miRNA. The assay is then performed by incubating the modified beads with the sample in the presence of a biotinylated DNA probe (signalling probe), complementary to a different region with respect to the capturing probe. Also in this case, the enzyme alkaline phosphatase (AP) conjugated with streptavidin is then incubated with the complex on the beads.
The three types of modified magnetic beads are then captured by an array of magnets on the surface of an array of graphite electrodes and the electrochemical detection is thus achieved through the addition, in each well of the array cell, of the AP substrate (α-naphthyl-phosphate). α-Naphthol produced during the enzymatic reaction is detected, on each electrode of the array, by using differential pulse voltammetry (DPV), leading to the quasi-simultaneous multianalyte detection of the three biomarkers.

REALIZATION AND CHARACTERIZATION OF GOLD NANOSTRUCTURES FOR AFFINITY BIOSENSOR DEVELOPMENT

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Over the last decade, the introduction of gold nanoparticles in sensor measurements has shown the possibility to obtain analytical systems characterized by high sensibility, selectivity, and reliability. This is due to a unique combination of electronic, optic, and catalytic features that, togheter with an easier synthesis, stability and nano-scale dimensions (diameter: 1-100 nm), lead to the realization of high versatile and miniaturized sensors. Furthermore, gold nanoparticles offer an extend area available for the immobilization of bioreceptor (antibodies, peptides, nucleic acids) and simple functionalization routes for the realization of biosensors.

In this work, gold nanoparticles were developed and characterized to realize electrochemical and optical biosensors for the determination of cancer biomarkers. After a morphological characterization (UV), various strategies of stabilization and functionalization with enzymes, antibodies and DNA fragments were studied.
Moreover, nanostructured surfaces were realized by modifying gold screen printed electrodes with gold nanoparticles, in order to increase the sensitivity of electrochemical biosensor with respect to traditional systems. For this purpose different functionalization strategies were studied: chemisorption, electrodeposition, electropolimerization with aniline. The stability and the electrochemical properties of these surfaces were studied by electrochemical impedance (EIS) experiments. Subsequently, in order to assess the utility of these architectures as DNA-sensing devices, a thiolated DNA capture probe sequence was immobilized onto the as-prepared surface . After hybridization with biotinylated target DNA, hybrids were labelled with sterptavidin-alkaline phosphatase and the electroactive enzymatic product was quantified by differential pulse voltammetry (DPV) as well as impedance spectroscopy techniques. Serigraphic single and 8 electrode array screen printed cells were employed for the realisation of the biosensor as well as transduction system.

MOLECULAR MODELING FOR RATIONALIZING BIOMIMETIC COMPOUNDS SELECTION: APPLICATIONS IN BIOSENSORS AREA

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A strategy to develop a new molecular modeling approach in order to rationalize generation of receptors by means of computational and programming techniques is proposed in this work. A protocol was established for design and identification of binding structures with biomimetic characteristics to be coupled with biosensor transducers, minimizing random combinatorial approach. The first step was to build a database of modified sequences using as backbone the tetrapeptide His-Glu-Pro-Ser mimicking acetylcholinesterase (AChE) binding site, found to have good experimental and simulated binding constants vs. pesticides[1].

In order to increase the degrees of freedom and structure flexibility, but preserving the verisimilar shape of AChE binding site key aminoacids (Ser200, His440, and Glu327) [2] the tetrapeptide was elongated inserting between the third and fourth position one or two aminoacids using all 20 natural ones. Moreover, positions of histidine and glutamic acid were swapped, obtaining respectively, 40 pentapeptides and 800 hexapeptides. Twenty-two organophosphates (OP) and five carbamates (CM) pesticides including all different OP and CM subclasses were chosen as target for simulation. AutoIt v3, a freeware BASIC scripting language for Windows tasks, was used to automate all steps. Using Openeye scientific software applications pesticides and peptides were fast minimized using Szybki, a tool designed to optimize molecules with the Merck Molecular Force Field (MMFF) in vacuum, solution and within a receptor (protein). To take into account the flexibility of structures[3], conformers were generated for all molecules by means of OMEGA module, a systematic high-throughput conformers generator by Openeye which uses higher quality force fields (MMFF and derivatives). Before calculating the binding score, a dedicated active site box for each peptide, defining the general space where pesticides are expected to bind, was designed resulting of a average volume of 6000Å^3 with an inner and outer contour respectively of 600Å^3 and 4000Å^3.

After these preliminary steps, Docking of each pesticides conformer in the peptide site and scoring were carried out with FRED (the protein-ligand docking program by Openeye) with a Gaussian shape fitting function to optimize the contact surface between the pesticides and the peptides. Typical docking time for FRED is a few seconds per target, for screening all database the computation time was of approximately 5 days. As an output file, FRED returns a hit-list of scored docked structures, from those results, considering the contribution of all pesticides, the average peptide conformers binding scores ranged from -300 to 100 expressed as arbitrary units with a coefficient of variation of around 20%. The best peptide in terms of binding score was EHWWPS. Only 4% of the peptides reached a score between −300 and −150 while the majority of peptides (90%) had total score between −150 and 0. The primary structure analysis of the 4% higher binding score peptides revealed that all were hexapeptides, the histidine or glutamic acid at first position were respectively 41% and 59% and % of aminoacids presence in the primary structure considering the third, fourth and their sum are reported in Figure 1.

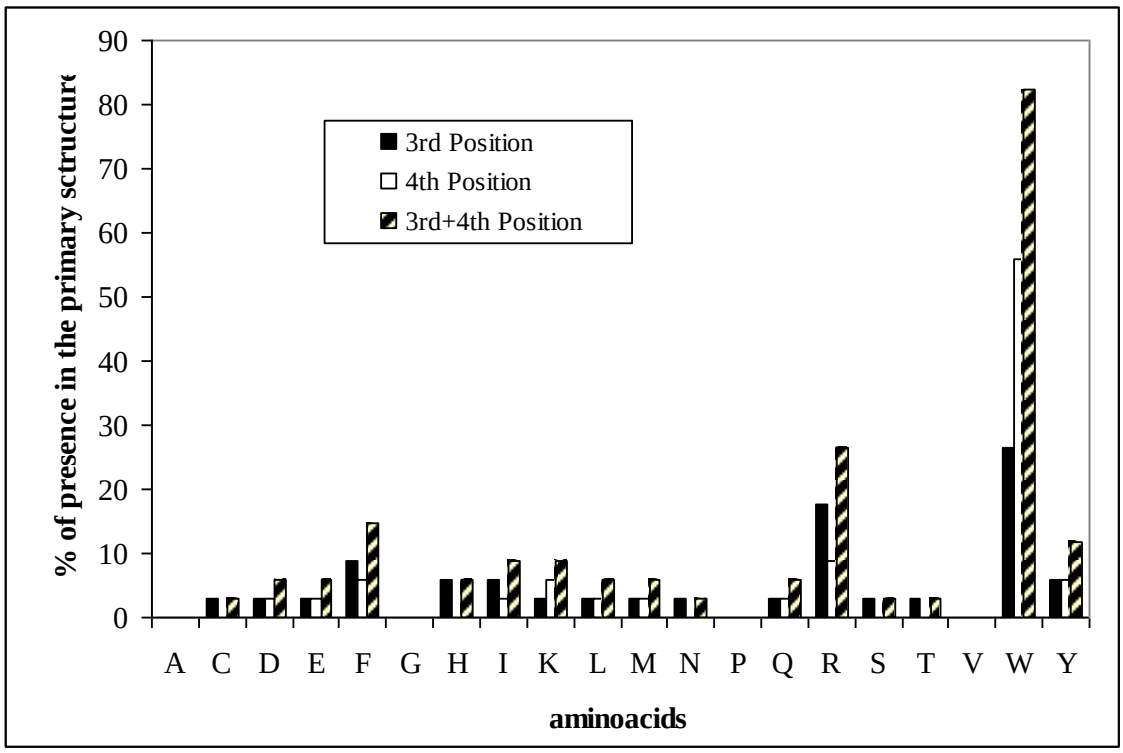

Figure 1. % of aminoacids presence in the primary structure in third, fourth and their sum in the 4% higher binding score hexapeptides.

Starting from these data an array of 5 sensors will be tested and the contribution of the primary structure to the experimental binding will be evaluated coupling to the biosensors transducers the following peptides: EHWWPS the best one, HEWWPS for understanding the swapping HE-EH position, HEWRPS to take into account the most repeated aminoacids W and R, EHSWPS for the contribution of serine, HEFRPS a sequence without W the most repeated aminoacid.

Acknowledgments
This work was supported by the 7th FRAMEWORK PROGRAMME Marie Curie Actions People IRSES N°230815 NANOSENS

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Bioinformatics for genosensors development. A case-study for single strand-DNA probes selection

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A post-PCR nucleic acid study by comparing bioinformatics results and experimental data from electrochemical sensors was made in order to rationalize the selection of single strand-DNA (ssDNA) probes using as case study the detection of two *Bonamia* species, protozoan parasite infecting haemocytes of several oyster species (*1*). Bonamiosis is mainly caused by *B. ostreae* and *B. exitiosa*, these species has been recently reported in *O. edulis* from Adriatic sea (*2*). In order to identify the proper *Bonamia sp.* a restriction fragment length polymorphism (RFLP) assay is necessary (official method) after a PCR reaction with genus specific primers. In case of polymorphism, genosensors can identify differences in amplicon primary sequence discriminating even single nucleotide polymorphism if suitable probes are selected (*3*). In this work molecular modeling approach was used to evaluate factors influencing hybridization between probes and PCR amplicons. 6 ssDNA probes (from 11 to 25 bases in length, 2 thiolated and 4 biotinylated) were selected within different regions of *B. ostreae* and *B. exitiosa* PCR amplicons (300 and 304 bases respectively) in order to discriminate between these parasite species.
In experimental phase, unmodified PCR products were captured at the sensor interface via sandwich hybridization with surface-tethered probes (T1 and T2) and biotinylated signaling probes (B1, B2, B3 and B4). The resulting biotinylated hybrids were coupled with a streptavidin–alkaline phosphatase conjugate and then exposed to a α-naphthyl phosphate solutions.
In computational phase, the secondary structure of all **ss**DNA was analyzed separately using the "mfold Web Server" modifying experimental conditions to 25° C and 1 M of Na^+. As a result, it was possible to simulate the ssDNA folding behavior obtaining information on the intramolecular hydrogen bonds that can reduce the availability of the target strand, thus lowering the hybridization efficiency. The hybridization of amplicon - probe was also considered using the "Dinamelt Web Server". Paired bases, position in which probes bind amplicons, number of hydrogen bonds formed and sequence gaps were used as descriptors. An empirical scoring function based on these modeling results was evaluated attending to: 1. number of hydrogen bonds broken and formed in the hybridization process, 2. variance in gaps for each sequence, 3. number of available bases. In the table below the experimental and simulated data are reported.

Probe	***Probe Bases no-binding ostreae***	***Probe Bases no-binding exitiosa***	***Probes combination***	***Ex-Os ratio of analytical Signal***	***Ex-Os ratio of empirical binding score***
B1	0	0	**T1-B1**	1.12	1.23
B2	0	7,8,9,10,11	**T2-B1**	1.21	1.08
B3	1,7,8,9,10,11	0	**T1- B2**	0.36	0.39
B4	1,5,6	0	**T2- B2**	0.72	0.48
T1	1	1	**T1- B3**	1.05	3.26
T2	0	0	**T2- B3**	1.87	1.82
			T1- B4	Not analyzed due to overlapping	
			T2- B4	1.68	1.13

Probes B1, T1 and T2 bound very well both *B. exitiosa* and *B. ostreae* amplicons. These results were expected since these 3 probes were chosen to bind conserved sites in both amplicon sequences. B2 was specially designed for binding *B. ostreae* with total complementarity. In computer simulation, probe B2 had only 6 out of 11 bases binding to *B. exitiosa* amplicon. Probes B3 and B4 targeted two zones of *B. exitiosa* sequence not conserved in *B. ostreae* having respectively 6 and 3 bases that did not bind *B. ostreae*.
Data displayed in table, shows a good convergence between analytical signals and empirical binding scores ratios. Experimental results ratio involving T2 were more favorable to *B. exitiosa* than those using T1 with the same biotinylated probes. This phenomenon can be justified because T1 has a base that do not interact with neither amplicons. The probes pair showing the highest deviation from experimental results was T1-B3. The probes pair T1-B4 was not tested because of a sequence overlapping. Probe B1 when used with both T1 and T2 had analytical signal and binding score ratios (10% - 20% aproximately) with a slight propension for *B. exitiosa*. Probe B2, as expected, had a very good response for *B. ostreae*, when used with T2 the *B. ostreae* signal and score ratios, were about 3 times higher than those of *B.exitiosa*. T2-B3 and T2-B4 gave analytical signal and binding score ratios (60% - 80% aproximately) favorable to *B. exitiosa*.
This work showed a convergence between analytical signal and simulated binding score, indicating the possibility to use bioinformatics for selection of ssDNA probes to be used in genosensors development.

Acknowledgement: this work was supported by the European Union within the project BIOMIMIC, no. FP7–PEOPLE–IRSES–2008-230849

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PHOTOSYNTHETIC REACTION CENTER EMBEDDED IN SUPPORTED PHOSPHOLIPID BILAYERS IMPLEMENTED IN OTFT BIOSENSORS

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To satisfy the demand for fast and smart analytical systems a great interest has been focused on the study and development of novel biosensing devices and several electrochemical and optical devices have been proposed [1, 2]. However, miniaturization, integration on flexible substrate, low-cost fabrication techniques, signal amplification and label-free detection are still open issues. Electronic transduction can open new perspectives for point-of-care diagnosis and treatment monitoring actuated by fast, sensitive, selective and reliable biosensors. Organic field-effect transistors (OFET) are presently blazing the trail for a low-cost and all printed technology platform for distributed intelligence, such as e-paper, electronic ID-tags, sensor stickers, etc. Such a technology should be robust and easy to manufacture and must operate at a power and voltage compatible with the energy sources available for low cost, portable flexible electronics. OFETs have already demonstrated the ability to detect numerous analytes in vapour systems, and the recent adaptation to liquid environments indicates their potential for biological detection [3, 4].
The most important aspect in the design of biosensors is the choice of an immobilization strategy ensuring that the biological activity of the immobilized biomolecule is retained. Novel bio-FET will be presented where the immobilization of the biological recognition element is obtained by means of the Photosynthetic Reaction Center incorporation into lipid bilayers, which can provide nearly native environment for biomolecules. The mechanism of electronic transduction will intervene by the exploitation of conformal changes and/or charge generation/re-distribution occurring upon the recognition process. Structural and morphological analysis has been performed to relate the conformational changes to the electronic response of the OTFT bio-sensor.

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AFFIBODIES AS AN ALTERNATIVE TO ANTIBODIES IN ELECTROCHEMICAL BIOSENSOR FOR HER2 DETECTION

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The goal of this work was to design a sandwich immunoassay with electrochemical transduction for the detection of a tumour marker, human epidermal growth factor receptor 2 (HER2), in complex matrices, using a simple target capturing step by antibody-functionalized magnetic beads.
HER2 (also known as neu, ErbB-2, ERBB2) stands for "Human Epidermal growth factor Receptor 2" and is a protein giving higher aggressiveness in breast cancers. It is a member of the ErbB protein family, more commonly known as the epidermal growth factor receptor family. The HER2 oncogene is a tyrosine kinase receptor that is over-expressed in approximately 20-30% of high-grade invasive breast carcinomas and has been shown to be a valuable prognostic indicator. HER2 positive cancer can be more aggressive and their status can predict response to targeting therapy such as chemotherapy. Knowing that a cancer is HER2 positive helps a medical team to select the appropriate treatment.
The assay is based on a sandwich format in which a primary monoclonal antibody anti-HER2 is coupled to protein A modified magnetic beads. The modified beads are then used to capture the protein from the sample solution and the sandwich assay is performed by adding a secondary monoclonal antibody anti-HER2 labelled with biotin. The enzyme alkaline phosphatase (AP) conjugated with streptavidin is then incubated with the complex on the beads. After, the modified magnetic beads are captured by a magnet on the surface of a graphite working electrode and the electrochemical detection is thus achieved through the addition of the AP substrate (□ naphthyl phosphate) and□ naphthol produced during the enzymatic reaction is detected using differential pulse voltammetry (DPV).
The potential to use affibodies in the assay for capture or detection of HER2 instead of antibodies have been investigated. The optimal conditions for affibodies as detection molecules have been explored to ensure that the best performance of the assay.

ERYTHROPOIETIN: A BIOSENSOR APPROACH

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Erythropoietin or EPO is a glycoproteic hormon of 165 aminoacids (MW about 30 KDa), belonging to hemopoietic growth factors. The total molecular mass depends on the glycosylation degree of the protein, which is quite variable, and originates form posttranslational modification, and represent the only difference between the different isoforms of human EPO, all biologically active, differing in the isolectric point (IP) in the range 3,8 e 4,7. The carbohydrates percentage is about 40& of the weight.
EPO is found in human serum and urine. Protein concentration are respectively in serum 2,5 pM, in urine 0,4 pM.
Recombinant EPO has been introduced in early '80 to treat patients i.e. with severe anemia and to reduce side effects other EPO produced in eukaryotes have been introduced and different analogs produced over years. Below a synthesis of them.

vs. EPO	I generation	II generation	III generation
Name	Epoetin α, β	NESP o darbepoetin α	CERA
a.a. sequence	Same a.a. sequence stessa of endogenous EPO	5 different a.a.	epoetin β modified with PEG
Glycosidic profile	Different glycosidic profile	iperglycosilted	
Half-life	Short half-life (8 h)	Half-life (48h)	half-life up to 130 h

Since 2004, when Epo alfa and beta patents were over other molecules have appeared such as Epoetin ω and δ*).*

EPO results in the prohibited list of World Anti-doping organization (WADA) and their abuse should be controlled in sport. Since 2000 at Sydney Olympic games, has been analyzed first in serum and then in urine for confirmation analysis, and since then direct analysis of EPO in urine should be mandatory since 2003, following WADA indications. Current analytical methods are based on electrofocusing, coupled to double blotting. Epo analysis is quite difficult since the molecule has relatively short half-life, numerous isoforms and many analogs are present on the market.
We describe the development of an optical immunosensor based on Surface Plasmon Resonace (SPR) for EPO detection in urine. The main analytical sensor parameters are described (i.e. sensitivity, selectivity, analysis time, matrix effect).

Acknowledgments: **World Antidoping Agency** (WADA), is acknowledged for financial support, with the project: Affinity-Based Biosensing (ABBs) for gene doping detection: a pilot study; Coordinator M: Minunni

VOLTAMMETRIC CHARACTERISTATION OF MICROBIAL COMMUNITY OF A MFC.

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Microbial fuel cells (MFCs) are devices that convert chemical energy to electrical energy through the catalytic activity of microorganisms (Allen and Bennetto 1993; Rao et al. 1976). In a MFC, electron donors are oxidized at an anode with concomitant production of carbon dioxide, protons, and electrons. The latter are transferred to an anodic electrode (Rabaey and Verstraete 2005). Because of their unique characteristics, MFCs are expected to have a wide range of applications such as wastewater treatment and sustainable energy generation.
Hence, research has focused on strategies to enhance the power output of the MFC devices, including exploring more electrochemically active microbes to expand the few already known electricigen families. However, most of the MFC devices are not compatible with high throughput screening for finding microbes with higher electricity generation capabilities. Here, we describe the development of a microfabricated MFC array, a compact and user friendly platform for the identification and characterization of electrochemically active microbes. The MFC array consists of 8 integrated anode and cathode chambers, which function as 8 independent miniature MFCs and support direct and parallel comparisons of microbial electrochemical activities.
In this study, we investigated the electrochemical of a *Pseudomonas sp*. strains that produce phenazine-1-carboxamide (PCN), a phenazine compound, at elevated levels together with biosurfactants.
The parallel analyses platform can greatly speed up research on electricigen microbes. Importantly, the array was fabricated using advanced fabrication approaches that can accommodate scale-up to massively parallel systems.

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