

# **Book of abstracts** The Ninth International Workshop

# on Biosensors

9th - 11th October, 2019 ELATI Hotel Erfoud, MOROCCO



2019

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# **SCIENTIFIC PROGRAM**

Wednesday		Thursday		
October 09th, 2019		October 10th, 2019		
08H30-09H15	H30-09H15 Registration &		Chair Christopher Brett	
	Poster display	09H00-09H50	PL2	
09H15-09H30	Opening	09H50-10H20	KN4	
Chair Palleschi		10H20-10H50	KN5	
09H30-10H20	PL1	10H50-11H10	OC 6	
10H20-10H50	KN1	11H10-11H20	DEMO PalmSens	
10H50-11H20	KN2	111110-111120	/ HTDS	
11H20-11H50	Coffee Break	11H20-11H50	CoffeeBreak and Poster Session	
	Session	Chair	Jaffrezic	
Chair V	adgama	11H50-12H10	OC7	
11H50-12H20	KN3	12H10-12H30	OC8	
12H20-12H40	OC1	12H30-12H50	OC9	
12H40-13H00	OC2	12H50-13H20	Meeting with	
13H00-13H20	OC3		Editors	
13h20-13h40	OC4			
13H40-14H00	OC5	13H20-14H30	Lunch and	
14H00-15H30	Lunch and		Poster Session	
	<b>Poster Session</b>	Chair Compagnone		
15H30-17H00	Visit of OASIS	14H30-14H50	OC10	
	BIOTECH-	14H50-15H10	OC11	
	NOLOGIE laboratory	15H10-15H30	OC12	
	Visit of Muse-	15H30-15H50	OC13	
	um of Fossils	15H50-16H10	OC14	
17H00	Departure to	16H10-16H30	OC15	
	Desert in Mer-	16H30-17H00	<b>CoffeeBreak and</b>	
-	Zouga		Poster Session	
KAR K			Errachid	
		17H00-17H20	0017	
		1/H20-1/H40		
		17H40-18H10		
		18H10-18H30	Tips for young	

Friday				
October11, 2019				
Chair Plaxco				
08H30-09H00	OC28			
09H00-09H30	KN6			
09H30-10H00	KN7			
10H00-10H30	KN8			
10H00-11H00	Coffee			
	Break			
Chair Kataky				
11H00-11H20	OC19			
11H20–11H40	OC20			
11H40-12H00	OC21			
12H00-12H20	OC22			
12H20-12H40	OC23			
12H40-14H30	Lunch			
Chair Arduini				
14H30-14H50	OC24			
14H50-15H10	OC25			
15H10-15H30	OC26			
15H30-15H50	OC27			
Amine, Brett, Palleschi				
15H50-16H10	Closing			
16H10-16H40	Coffee			
	Break			
20H	Diner			







Diner

**20H** 









.



Pleneary conferences

# PL-01



#### COUNTING MOLECULES, DODGING BLOOD CELLS: CONTINUOUS, REAL-TIME MOLECULAR MEASUREMENTS DIRECTLY IN THE LIVING BODY

Kevin W. Plaxco, University of California, USA

The availability of technologies capable of tracking the levels of drugs, metabolites, and biomarkers in real time in the living body would revolutionize our understanding of health and our ability to detect and treat disease. Imagine, for example, a dosing regime that, rather than relying on your watch ("take two pills twice a day"), is instead guided by second-to-second measurements of plasma drug levels wirelessly communicated to your smartphone. Such a technology would likewise provide clinicians an unprecedented window into organ function and could even support ultra-high-precision personalized medicine in which drug dosing is optimized minute-by-minute using closed-loop feedback control. Towards this goal, we have developed a biomimetic, electrochemical sensing platform that supports the high frequency, real-time measurement of specific molecules (irrespective of their chemical reactivity) in situ in the bodies of awake, freely moving subjects.

# PL-02



# BIOCHEMICAL INTERACTIONS AT LIQUID-LIQUID INTERFACES.

<u>R.Kataky</u>\*, R. Campos, P.Lopes, Y.Bunga, B.Silwane, N.Ntolo Department of Chemistry, Durham University, Durham, United Kingdom, DH1 <u>3LE</u> ritu.kataky@durham.ac.uk

Biological interfaces are 'soft' and 'electrified'. An electrified liquid-liquid interface, (ITIES: Interface between Two Immiscible Electrolyte Solutions), can be used as a biomimetic interface to study both fundamental aspects and practical applications. The following areas of research will be discussed:

- 1. Interactions of drugs :
  - a. The interaction of a drug with acute phase proteins in serum can influence their bioavailability and modify the function of biomolecules. Furthermore, the chiral purity of a drug plays an important role, since different isomers have distinctive clinical effects. A micro liquid-liquid interface was effectively used, for quantifying drug-protein interactions.
  - b. Several neurodegenerative diseases are caused by amyloidogenesis, which occurs through the accumulation of amyloid beta  $(A\beta)$  peptide and its aggregates at lipid interfaces. Beta amyloid aggregation and interactions of  $Cu^{2+}$  have been investigated and modelled. The effects of a multifunctional peptidomimetic inhibitor (P<sub>6</sub>) ,based on a naturally occurring, metal chelating, tripeptide and an inhibitor of A $\beta$  aggregation is studied.
- 2. Free-standing bilayer membranes are of fundamental biological significance. Techniques for studying the interactions of important mitochondrial membrane associated molecules, such as Ubiquinone-10 (UQ10) and  $\alpha$ -tocopherol (Vitamin E), are presented.
- 3. Resonance Enhanced Impedance Spectroscopy, a relatively new technique, is shown to be suitable for probing nanoparticle effects on lipid membranes, in real time.

4.

#### **References:**

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- 2. P.Lopes; R. Kataky, Anal. Chem. (2012), 84(5), 2299–2304.
- 3. C.N.N Ntolo and R. Kataky, Electrochem Comm, (2017), 7765-70,
- 4. Y.Bunga, R.Kataky, Sensors and Actuators B, Chemical 290 (2019) 140-146

# Keynote lectures

# <u>KN-01</u>



#### ELECTROCHEMICAL DNA NANO-BIOSENSORS DEVICES FOR DETECTION OF DRUG RESISTANCE

Hafsa Korri-Youssoufi, University Paris-Sud, France

# <u>KN-02</u>



#### MICROBIAL FUEL CELLS: ELECTROACTIVE ANODIC BIOFILMS FOR ELECTRICAL ENERGY PRODUCTION

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Microbial fuel cells (MFCs), as a potentially sustainable biotechnology, can mediate the direct conversion of organic matter from wastewater into electricity using bacterial biofilms as biocatalysts1. Bacteria capable of this reaction - called Electroactive Bacteria (EAB) - can use different mechanisms to transfer the electron to the anode: an indirect electron transfer via redox shuttles such as flavin as in Shewanella2 or a direct electron transfer via c-type cytochrome as in Geobacter3. One of the critical point in MFCs is to understand the mechanisms involved in the competition between electroactive bacteria (EAB) and other bacteria4. We formulated the hypothesis that the hydrodynamic force plays a major role in the biofilm formation and the selection of EAB on the anode. Firstly, MFC reactors allowing to have a control of the hydrodynamic force on the anode were designed and made. Then the evolution of the biofilm formation under different shear stress were compared together and with a classical MFC without shear stress. The microbiological structure was studied by DNA analysis and sequencing, the percentage of coverage was studied by fluorescence microscopy, and the electrical characteristic were followed by linear and cyclic voltammetry. The results show that the proportion of specific EAB such as Geobacter was higher, up to 30% as opposed to a lower shear stress (less than 1%). Then, the effect of shear stress on microbial selection during biofilm growth was studied. These results confirm the previous conclusions: specific EAB are selected when shear stress is higher. This work demonstrates the major role of shear stress in biofilm formation and could be a way to control the selection of EAB. This factor should be taken into account in the architecture and implementation of the reactors.

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- 4. Paitier, A. *et al.* Microbial fuel cell anodic microbial population dynamics during MFC start-up. *Biosens. Bioelectron.* **92**, 357–363 (2017).

#### Acknowledgements:

Authors greatly acknowledge PHC Maghreb 2019 project N°41382WC for its support to the realization of this work

# <u>KN-03</u>



#### A CO-OPERATIVE m*HEALTH* ENVIRONMENT TARGETING ADHERENCE AND MANAGEMENT OF PATIENTS SUFFERING FROM HEART FAILURE

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HEARTEN develops an ICT collaborative platform that empowers the HF patients to achieve sustainable behavior change regarding their adherence (medication, nutrition and physical activity) and the ecosystem actors (healthcare professionals, caregiver, nutritionist, physical activity expert, psychologist) to be engaged, and enhance the patient's wellbeing. The target population is patients who have been diagnosed with HF (Framingham criteria) and belong to New York Heart Association (NYHA) Functional Classification II-IV, with continuous symptoms and recent hospitalizations.

The main research activities of the HEARTEN project focus on the identification and validation of specific breath/saliva biomarkers, the development of breath and saliva biosensors integrated to the smartphone and the patient's cup respectively for monitoring the patient's medication adherence, and the application of data mining/knowledge management techniques for discovering and revealing interesting and useful patterns of patients' adherence and HF status.

The key elements of HEARTEN are: (i) the development, application and validation of analytical chemistry methods for HF biomarker determination in saliva and breath, (ii) the development and characterization of the breath/saliva biosensor silicon chips (design, fabrication, characterization) and the measurement device that digitalizes the sensing outcomes, (iii) the selection and utilization of appropriate sensors that measure real-time vital signs indicative of HF progress (electrocardiography, heart and respiratory rate, blood pressure, physical activity body temperature and weight), (iv) the development of mHealth apps which collect the sensor's/biosensor's and patient data and enable the ecosystem actors tight collaboration, (v) the development of a Knowledge Management System (KMS) that exploits and analyses real patient data coming from multiple sources (sensors, bio-sensors, clinical data, personal data) and extracts useful information and knowledge related to patient NYHA class, patient NYHA class change, adherence, risk for adverse events (relapses) and risk for death, alert for medication, physical activity and nutrition, (vi) the development of a Dynamic Patient Communication Protocol (DynPCP) for providing alerts, suggestions guidelines, trends and predictive models to the patient, (vii) the integration of HEARTEN platform in the multi-functional Cloud environment for supporting and ensuring the communication and collaboration of diverse technologies, (viii) the conduction of a pilot study in two different University hospitals (University of Pisa, School of Medicine and Virgen del Rocío University Hospital) to validate the HEARTEN platform, evaluate the specific life quality indicators and health-behavioral changes, value the benefits, assess the cost reduction expected after its adoption and drive the market to its wide scale deployment.

#### Acknowledgements

We acknowledge the funding through the European Union's Horizon 2020 research and programme entitled HEARTEN and KardiaTool under grant agreement No 643694 and 768686.

### <u>KN-04</u>



#### APPLIED BIOSENSING FOR THE MEDICAL INTERFACE

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Remarkable advances in biosensor transduction chemistries and especially the use of nanomaterials has given both greater biophysical insight and performance enhancement. However, the biomedical sample environment presents an altogether different type of problem – the need for sustained operational stability in biological samples that are either actively hostile to an intrusive device or which project active cellular and colloidal components on to the device surface through active surface deposition. The tackling of surface biocompatibility demands adaptation of biomaterial approaches<sup>1</sup>. Our work has centered on quite standard chemistries for amperommetric sensors, but emphasised the materials imperatives for senors. Here, barrier constructs for stable implanted sensors will be presented, and the how such devices during continuous monitoring enable interpretation of physiological changes. Examples will be given of monitoring of glucose, lactate and O<sub>2</sub> - quite basic parameters, but still of sustained importance in Medicine and not fully understood. Comparative results using microdialysis will show the conundrum presented by the tissue/blood duality as biochemically distinct entities. Results of ancillary approaches will also be presented, using recess tip electrodes to create controlled diffusion barriers, fluid flow tissue expansion to reduce fouling and temperature addressable poly(N-isopropylacrylamide) as a possible means of modulating sensor barrier permeability and surface organisation. A textile ion selective electrode with ready applicabity for sweat monitoring will be presented to demonstrate potential future use of alternate biofluids for trend monitoring.

#### Reference

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# <u>KN-05</u>



#### NOVEL MATERIALS FOR ELECTROCHEMICAL BIOSENSING OF NUCLEIC ACIDS

Ilaria Palchetti, University of Florence, Italy

In comparison with other methods, electrochemical approaches for bioanalytics are well known as simple and efficient tools due to the merits of low-cost instrumentation, fast operation and favorable performance, and have drawn considerable attention and interest in recent years. Indeed, electrochemical genosensors have been intensively studied due to their potential for nucleic acid testing, as a result of their appropriate sensitivity, multiplexing capability, and small amount of sample required. However, the catalytic activity, the electron transfer and chemical reactivity of the electrode material as well as the electrode surface area itself, can affect the biosensor sensitivity and selectivity. Thus, the choice of the electrode material and architecture should be carefully evaluated, in order to find the optimal biosensing strategy.

### <u>KN-06</u>



### SENSING OF CANCER BIOMARKERS USING NANOMETRIC E-bioplatforms

Noureddine Raouafi University of Tunis El Manar, Tunis, Tunisia

According to WHO, cancer is the second leading cause of death worldwide and thus should be reliably diagnosed at early stages preferably using noninvasive tools. Besides, protein cancer biomarkers, microRNAs (miRNAs), which are short non-coding single-stranded RNA oligonucleotides, approximately 18-24 bases in length are among leading cancer biomarkers. They post-transcriptionally regulate about 40% of all protein-encoding genes. In the last decade, they have been extensively reported as the clinical potentially relevant biomarkers for cancer diagnosis and/or prognosis form human body fluids avoiding invasive procedures. Their aberrant expression profiles (i.e. up- or down-regulation) have been demonstrated in numerous human cancers. In this presentation, we will show several detection concepts based on enzymatic amplification to electrochemically detect very low concentrations miRNAs present in human tissues and blood sera. To achieve these goals, we modified disposable electrodes with gold nanoparticles for tethering the DNA or RNA detection probes to the electrode surface and benefiting from gold nanoclusters signal amplification. The target is detected based on i) a competition with a biotintagged target, ii) p19 specific viral protein recognizing the RNA/RNA homoduplex iii) S9.6 antibody that recognized the DNA/RNA heteroduplex and iv) producing electrochemically active species. The electrochemical readout was achieved using horseradish peroxidase (HRP) linked to the proteins, which catalyzes the reduction of hydrogen peroxide in presence of hydroquinone producing electrochemically active benozquinone. Furthermore, we also developed a dual signalon fluorescence/electrochemical readout bioplatform to assess the levels of miRNAs were the enzymatically generated substance is electrochemically and optically responsive. All the bioplatforms were extremely sensitive and selective allowing the detection of the target oligonucleotide in complex media such raw total RNA extract for cancer cells and blood sera from cancer patients.

# <u>KN-07</u>



# ELECTROCHEMICAL NANOBIOSENSORS FOR DIAGNOSIS AND MONITORING OF DISEASES.

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Electrochemical nanobiosensors have proven potential use as diagnostic devices. Novel properties and functions of nanomaterials coming from their dimensions, differ from the concomitant bulk materials counterparts [1]. Rational design of biological structures and their assembly with the nanomaterials allows on-demand development of nanobioengineered platforms with improved properties and outstanding performance. In this context, new nanobiotechnology-enabled medical devices aim to provide convenient real-time diagnosis of diseases, closer to the patient, with respect to conventional technologies. Whereas nanobioprobes achieve a high response to very small targets in practical conditions, they are used to develop superior sensitive and specific assays, with reduced time-scale of testing and minimal requirements of samples volume. Diagnostic tests and devices based on biosensors are being increasingly exploited as valuable alternatives to standard laboratory instrumentation for clinical diagnosis, allowing simple, inexpensive and point-of-care testing systems. Biosensors are usually designed to be highly selective, sensitive, fast, portable, and easy to operate. They can be reusable, affordable, generally having responses that correlate to different analyte concentrations.

This talk is aimed to discuss novel electrochemical nanobiosensors that have been developed in our group for diagnosis and monitoring of pathogens and diseases. It will highlight innovative approaches regarding: i) Biosensors for the specific and highly sensitive pathogen detection, in a simple format [2], ii) Novel superior, hybrid nano(bio)platforms for the development of electrochemical (bio)sensors of improved performance [3]; and iii) Differential nanogenosensors for diagnosis of Zika virus and its discrimination among related viruses such as dengue and chikungunya. The talk will demonstrate the enormous potential of nanobiosensors for tackling real problems in today's world and will highlight their opportunities for multiple applications in clinical diagnosis and monitoring.

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# <u>KN-08</u>

#### PORPHYRINOID BASED SENSOR ARRAYS

C. Di Natale, R. M. Capuano, G. Magna, D. Monti, S. Nardis, M. Stefanelli, and <u>R. Paolesse</u>

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The development of smart and reliable chemical sensors has become increasingly important for several application fields, ranging from medical to environmental applications. A chemical sensor is in fact a device that transforms chemical information, ranging from the concentration of a specific sample component to the total composition analysis, into an analytically useful signal. Porphyrins are useful materials for chemical sensors development, since in these devices the porphyrins play the role of receptor, the component that interacts with the chemical environment and for this reason it mostly influences the performances of the device. Since 1995 we have been involved in the preparation of different kind of porphyrin based chemical sensors, for the detection of analytes both in liquid and in the gaseous phase. Porphyrins have demonstrated to be versatile receptors, able to detect a wide range of analytes by a manifold of different sensing mechanisms.<sup>1</sup> Moreover, this feature makes possible to compare the performance of different transduction principles. The selectivity pattern of porphyrin based chemical sensors can be tuned at the synthetic level, and it has been exploited for the development of sensor arrays. For some similarities of this approach with the working mechanism of natural olfaction led to name these sensor arrays as "electronic noses". The rational of the sensor arrays approach in comparison with natural olfaction will be discussed, together the results obtained in the application of these devices.

#### References

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# Oral communications

#### IMPLANTABLE BIOFUEL CELL, PAST, PRESENT AND FUTURE

#### Abdelkader Zebda,

#### University Grenoble Alpes, SyNaBi, TIMC-IMAG/CNRS/INSERM, UMR 5525, F-38000, Grenoble, France

In the past few decades, we have witnessed tremendous development in electronics, micro- and nanofabrication, and wireless technology which have greatly enhanced the quality and efficacy of healthcare as well as life-science research

A. Medical Devices (IMDs) are used to improve healthcare, aiding or delivering the functions of certain malfunctioning organs have been sustained for years. However, to ensure proper operation, most IMDs need to rely on a permanent and sufficient power supply, thus numerous power sources for IMD have been widely investigated in the last decades. The existing technology is to utilize a rechargeable lithium battery, but a battery with an acceptable lifetime and size is only indicated for implanted devices consuming a few tens of microwatts [2]. In this context biochemical energy harvesters, also called **biofuel cells, that convert** the energy stored in chemical bonds into electrical energy are very promising. In medical implants, a biofuel cell generates the power by complementary chemical reactions at a pair of electrodes. Oxidation occurs at the anode electrode and reduction takes place at the cathode [3]. Chemical reactions are accelerated by the participation of biocatalysts (enzymes). Due to its omnipresence in body fluids, glucose is the most commonly used fuel in biofuel cells [4,5]. This conference is focused on highlighting the recent progress on implanted biofuel cells technologies, the medical and physiological aspects related to their operation inside life body. The review discuss the challenge of sup-power implantable medical devices, the limitations of lithium batteries technology and why implantable biofuel cells can be a promising alternative. Besides, the conference on the issue of implanting a biofuel, the biocompatibility and biofouling problems related to their interaction with the life body. Moreover, the review discuss on the availability of the substrates inside body and how the, and how the biofuel cells can be long-lasting and ideally become symbiotic with the body [1].

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c Zebda *et al*. Mediatorless high-power glucose biofuel cells based on compressed carbon nanotubeenzyme electrodes Nat. Commun. (2011) 2: 370

d El-ICHI-et al. Bioelectrodes modified with chitosan for long-term energy supply from the body. Energy Environ Sci. (2015); 8 (3): 1017-26

e El-ICHI et al. Remote wireless control of an enzymatic biofuel cell implanted in a rabbit for 2 months

Remote wireless control of an enzymatic biofuel cell implanted in a rabbit for 2 months. Electrochimica Acta 269 (2018) 360e366

#### APPLICATION OF LANGMUIR-BLODGETT (*LB*) FILMS OF SELECTED BIS(BITHIENYL)METHANE DERIVATIVES FOR SURFACE IMPRINTING OF HUMAN SERUM ALBUMIN

K. <u>Gołębiewska<sup>1</sup></u>, A. Szymańska<sup>1</sup>, P. Borowicz<sup>1</sup>, S. Shao<sup>2</sup>, F. D'Souza<sup>2</sup>, K. Noworyta<sup>1</sup>

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Surface imprinting become a highly attractive field for preparation of macromoleculesimprinted polymer films applied in chemosensing<sup>1</sup>. It allows for preparation polymers with fitting binding sites for large target molecules of the analyte.

This presentation describe method allowing easy formation of thin films of the polymers imprinted with protein with controlled film thickness. In this work human serum albumin (HSA) was used as an analyte. HSA is a biomarker of numerous diseases<sup>2</sup>. Langmuir technique allows to create self-assembly protein - HSA template with thiophene derivatives functional monomers at the air-water interface. The resulting of pre-polymerization complex is then transferred onto solid substrate via Langmuir-Blodgett technique. Fabricated Langmuir-Blodgett films were studied by UV-vis, PM-IRRAS and AFM techniques. Thickness of the final ultrathin film is easily controlled from sub-nanometer to dozens of nanometers. The studies confmired formation of HSA-bis(bithienyl)methane derivatives complex and its successful transfer onto solid substrates.

Subsequently, the final complex were stabilized by electrochemically polymerization. In next step the HSA-template was extracted. Finally the prepared polymer film with "molecular imprints" were used as the recognition part in the extended-gate field-effect transistor (EG - FET) chemosensor. Influence of the film formation and deposition parameters on the chemosensor parameters have been studied.

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Fig. Concept of chemosensor preparation at the air-water interface

#### APTASENSORS THAT DISTINGUISHES SUB-TYPES OF INFLUNEZA A VIRUSES

#### Penmetcha Krishna Rajendra Kumar

Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba Science City, Ibaraki 305-8566, JAPAN

Rapid influenza diagnostic tests, RIDTs, for evaluating influenza antigens have been widely used in clinical diagnosis of influenza viruses since their inception more than three decades ago. These RIDTs are adopted quickly in clinics because test results provided under 20 minutes, simple to use and suitable for point of care testing. However, issues related to their sensitivity of RIDTs have been known for decades and these issues gained further attention when sensitivity reduced for diagnosis of sub-types of influenza A viruses (H5 and H7) and also influenza B viruses compared to other sub-types of influenza viruses [1]. Furthermore, the current RIDTs are suitable for detecting broadly influenza A and B viruses but not suitable for differentiating subtypes of influenza A viruses. Considering pathogenesis and severity of infections caused by H5 and H7 sub-types of influenza A viruses, it is important to develop the next generation RIDTs that can differentiate all sub-types of influenza A viruses including, highly pathogenic sub-types (H5 and H7).

In the past, several high affinity and specific aptamers have been isolated against many viral proteins, including surface proteins of human pathogenic viruses. Interestingly, some of our selected aptamers are able to distinguish very closely related antigens of HIV and HSV viruses [2, 3] and influenza viral antigens [4-7]. I plan to present, at the conference, our recent studies on exploring our sub-type specific aptamers in developing the next generation RIDTs that distinguishes sub-types of major influenza A viruses.

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#### A MINIATURIZED ALL-SOLID-STATE ION-SELECTIVE ELECTRODE BASED ON A CARBON BLACK TRANSDUCER FOR SODIUM DETECTION IN SWEAT

V. <u>Mazzaracchio<sup>1</sup></u>, A. Serani<sup>1</sup>, D. Moscone<sup>1</sup>, F. Arduini<sup>1</sup>

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One of the hot topics in bioanalytical field is the development of non-invasive, and easy to use sensing devices for biomarkers monitoring. Highly improved by the use of novel technologies, point of care devices have gained an important role in the continuous and ubiquitous monitoring of people health state [1].

Among the big plethora of point of care sensors, the ones exploiting electrochemical techniques deserve a primary role: the use of potentiometry, amperometry and voltammetry allows for the fabrication of deliverable, disposable, based on a minimal sample requirement and low-cost sensors.

Among these, potentiometric ion-selective electrodes (ISE) with internal solid contact (SC-ISE) have attracted high attention in this decade due to their specificity and easy fabrication process. Compared to classic ISE, SC-ISE exploits conductive polymers and nanostructured materials for ionic transduction, to replace the liquid contacts of the classic ISE, and a flexible substrate for sensors fabrication, i.e. polyester, polyethylene or paper [2]. Moreover, an ionophore-containing membrane is used for the analyte detection.

Following these principles, we developed a miniaturized all-solid state ion-selective electrode, coupled with a portable potentiostat palmsens 3, for  $Na^+$  detection in sweat sample (Figure 1). The sensor consists of a low-cost fabricated screen-printed electrode (SPE), modified with Carbon Black (CB), in order to effectively convert the ionic signal through the ion-selective membrane into an electronic signal. Two different membranes were exploited: the first one, located onto the CB-modified graphite working electrode surface, contains a sodium ionophore and allows for the selective detection of the ion. Moreover, a reference membrane is placed onto the Ag/AgCl reference electrode to guarantee the acquisition of a stable and accurate potential. Both were prepared in an organic solvent, namely THF and methanol, and drop-casted onto the electrodes.

Firstly, several parameters for the sensor optimization were studied, namely volume of CB, volume of selective and reference membrane and conditioning process time.

After, a calibration curve was obtained by potentiometric measurements of standard solutions, with a linear response in the range between  $10^{-3}$  M and 1 M and a regression equation equal to y = 0.068 x + 0.262,  $R^2 = 0.984$ .



Figure 1. Picture of the developed sensor

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#### BIOELECTRONICS SURFACE BASED ON CONJUGATED DI-BLOCK POLYTHIOPHENE CONJUGUATED MANNOSE FOR LABEL-FREE BACTERIA DETECTION

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Development of effective pathogen detection systems is critical in medicine, food safety, public health, and security. In this study, we develop a new label-free bioelectronics surface based on conjugated amphiphilic di-block copolymer for the detection of whole bacterial cell. Escherichia coli (*E. coli*) where is selected as target as their presence is the signature of sample contamination. This biosensor is based on a co-polythiophene modified with oligo-ethylene glycol and hexyl chain in lateral positions, as transducers and mannose carbohydrate as sensing layer (see scheme).

The biolayer is formed through micro-emulsion method where mannose immobilization is favored by hydrogen bonding of oligo-ethylene glycol of co-polythiophene. Direct deposition of the bio-composite by drop casting on electrode on the surface leads to obtain a sensitive layer without any pre-treatment. Detection of *E.-coli* was studied and evaluated by electrochemical impedance spectroscopy (EIS), optical measurements and scan electron microscope (SEM) with the composite and compared with non-modified copolymer.

We demonstrated that detection of *E-Coli* is obtained with the composite bearing mannose with dynamic range from  $10^3$  CFU/mL to 106CFU/mL. The bio-surface also showed satisfactory selectivity in discriminating gram-negative *E. coli* from gram-positive bacteria. The entire biosensor provides good targeting ability toward *E. coli* pili due to the selectively binding to the FimH lectin unit of mannose.

The developed biosensor is compatible with low-cost fabrication techniques and could be extended to the design of EIS biosensors for highly sensitive and rapid detection of other desired bacteria by selecting the targeting carbohydrate.



9th - 11 th October, 2019 Erfoud, MOROCCO

#### THE SONOGEL-CARBON-PEDOT MATERIAL: AN INNOVATIVE BULK MATERIAL FOR SENSOR DEVICES

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Conducting polymers have widely attracted attention in the last decades due to their good electrical properties, like high conductivity, which are suitable for electroche-mical applications, such as supercapacitors, batteries and sensors. Particularly, poly-(3,4-ethylenedioxythiophene) (PEDOT) has currently been employed in (bio)sensors because of its high stability and reduced band-gap, among other characteristics [1,2]. Despite of several advantages regarding the use of these devices, their stability is an interesting topic, which generates much controversy, mainly due to the degradation of the conducting polymer coating after many successive measurements [3].

In this work, a ceramic material based on the Sonogel-Carbon material [4] and PEDOT is obtained by sol-gel route. The fabrication process was assisted by .a green synthesis technology: high power ultrasound. Hence, EDOT was included in the silicon oxide network and promoted to PEDOT by sonocatalysis. The electrical conductivity of final material is increased by the presence of the conducting polymer in the matrix. With the objective of maximizing its electrochemical response, the optimization of the synthesis conditions is performed by a factorial experimental design.

The efficiency of the Sonogel-Carbon-PEDOT material as transducer in sensor devices is demonstrated taking into account repeatability and reproducibility studies (RSD<3%) for ascorbic acid (AA), selected as benchmark analyte: sensitivity = 319.8  $\mu$ A·mM<sup>-1</sup>·cm<sup>-2</sup> and limit of detection = 6.42  $\mu$ M. The easy and gentle mechanical polishing of the surface increases the lifetime of the sensor, minimizing fouling phenomena. In addition, the Sonogel-Carbon-PEDOT electrode was successfully applied to the quantitation of AA in biological and pharmaceutical samples, obtaining good recovery percentages (95–105%). Finally, the material obtained was characterized by different techniques: SEM, EDS and Raman spectroscopy. The reported method of synthesis based on high power ultrasound is very versatile, offering the possibility of obtaining many other interesting electrode bulk materials. As far as we are concerned, this is the first time that this kind of bulk material is reported [5].

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#### GREEN AND FAST SYNTHESIS ROUTE OF (BIO)SENSING MATERIALS USING A HIGH POWER ULTRASOUND PROBE: RECENT ACHIEVEMENTS

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Since the past few decades, the term Green Chemistry, firstly introduced by Anastas at the end of 90's and defined as 'the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances' [1], has been widely extended and applied for materials syntheses. Ultrasound and microwaves are considered to be the base of the most powerful, ecological and interesting technologies developed for this purpose [2]. Specifically, high power ultrasound, when is applied directly to a solution using a probe, leads to the sonochemical effect. This usually arises from acoustic cavitation which generates enough kinetic energy to drive reactions to completion and releases short-lived, high-energy chemical species into solution after the implosive collapse of bubbles. As a consequence, this process is capable of providing very high temperatures (4500-5000 K is typical) and pressures ( $\approx 1000$  atm) in extremely short times (in the range of microseconds), what makes ultrasound very attractive for syntheses purposes. This fact is remarked by its exciting advantages: i) environmentally friendliness, ii) very low energy requirements, iii) drastically reduced time of synthesis: from days/hours to few minutes, and iv) simple and v) low cost instrumentation compared to other technologies.

In the present work, recent achievements of our research group when applying high power ultrasound for synthesizing (nano)materials for (bio)sensing are reported. Firstly, 'the use of sonochemistry for obtaining nanomaterials with specific features, especially metal (Au) and metal oxide nanoparticles (Fe<sub>3</sub>O<sub>4</sub>), is described. Secondly, the green synthesis of nanostructured molecularly imprinted polymers is exposed [3]. Finally, a short description of the synthesis of Sonogel-Carbon-based electrode materials is also given [4]. In all cases, structural characterization of the (nano)materials and their analytical application are included.

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#### HUMAN CYTOCHROME P450 (CYP1A2)-DSDNA INTERACTION *IN SITU* EVALUATION USING A DSDNA-ELECTROCHEMICAL BIOSENSOR

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Cytochromes P450 are ubiquitously distributed enzymes that were discovered about 50 years ago. They are hemoproteins encoded by a superfamily of genes, possess high complexity and display a large field of activity being involved in the biotransformation of drugs, the bioconversion of xenobiotics, the metabolism of chemical carcinogens, the biosynthesis of physiologically important compounds, as well as in the degradation of herbicides and insecticides.

Human cytochrome CYP1A2 is one of the major hepatic cytochrome P450s involved in many drugs metabolism, and chemical carcinogens activation.

The electrochemical methods, such as pulse techniques, were suitable for investigating CYP1A2-dsDNA interaction [1], since they presented higher sensitivity, and allowed the *in situ* electrochemical detection, for very low concentrations not possible using other available methods, of the adsorbed CYP1A2-dsDNA interaction products formed on the GCE surface.

The dsDNA-electrochemical biosensor showed that CYP1A2 interacted with dsDNA causing its condensation and DNA oxidative damage. The poly[G]- and poly[A]-electrochemical biosensors confirmed that CYP1A2 interacted with dsDNA preferentially at guanine-containing sequences, leading to DNA oxidative damage, and enabled the detection of the 8-oxoGua biomarker.

The CYP1A2-dsDNA interaction showed the occurrence of dsDNA condensation in incubated solutions and dsDNA oxidative damage using the dsDNA-electrochemical biosensors, confirmed by UV-Vis spectrophotometry.

The molecular mechanism involved in the CYP1A2-dsDNA interaction, by which the enzyme CYP1A2 interacted with dsDNA causing oxidative damage and mutagenicity, in a time-dependent manner, was clarified and is now better understood.

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#### MODIFIED GRAPHITE CERAMIC ELECTRODE WITH MULTI-WALLED CARBON NANOTUBES AND GOLD NANOPARTICLES FOR SIMULTANEOUS DETERMINATION OF PURINE DERIVATIVES

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Purine derivatives such as uric acid (UA), xanthine (XA) and caffeine (CA) are compounds with very similar chemical structures<sup>1</sup> that play a key role in living organisms. Their concentrations in bodily fluids such as blood serum and urine can provide crucial information about pathological conditions that may arise, making them sensitive indicators  $^{1-3}$ . In this study, we develop a novel modified graphite ceramic electrode with multi-walled carbon nanotubes-COOH-rich and gold nanoparticles (MGCE/MWCNT-COOH-AuNPs) for the simultaneous determination of UA, XA, and CA. This electrode was successfully characterized using transmission electron microscopy, Fourier-transform infrared spectroscopy, field-emission environmental scanning electron microscopy, and energy-dispersive X-ray spectroscopy. The proposed modified electrode displays three sharp, stable and continuous well-distinguishable oxidation peaks towards electrooxidation for UA, XA, and CA. The calibration curves were linear up to 275, 300 and 1500 µM with a limit of detection of 50, 63 and 354 nM for UA, XA, and CA, respectively. The analytical performance of the MGCE/MWCNT-COOH-AuNPs was successful in the simultaneous detection of the three purine derivatives in real samples such as human serum and urine, with recoveries of 98.1 - 102.6%. The catalytic currents were further challenged with a very similar purine molecule known as theobromine (contains one less methyl group than caffeine). Results revealed a clear peak separation for theobromine without any interference. Overall, this MGCE/MWCNT-COOH-AuNPs exhibited an extremely promising platform for the future development of sensitive electrochemical sensors as clinical diagnostic tools for purine derivatives in real samples.

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# DNA-ELECTROCHEMICAL BIOSSENSOR APLICATIONS IN THE INVESTIGATION OF DRUG-DNA INTERACTION MECHANISMS

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A DNA-electrochemical biosensor consists of a device in which the DNA is immobilized on the electrochemical transducer surface to detect specific binding processes with different molecules, to monitor changes in the DNA conformational structure after interaction with these molecules, and the appearance of DNA base residues and their oxidation products, the biomarkers of DNA oxidative damage.

The DNA-electrochemical biosensors in the investigation of drug-DNA interaction have enabled to elucidate the drugs implications in DNA structural modifications, and the mechanistic and cytotoxic aspects of their physiological action.

The interaction between different drugs, such as the antileishmanial miltefosine, the antidiabetic metformin, and the anticancer monoclonal antibody nivolumab, with dsDNA, in incubated solutions using differential pulse voltammetry, UV-Visible spectrophotometry, and/or gel electrophoresis, was investigated. The drugs-DNA interaction was also evaluated using the dsDNA-, poly[G]- and poly[A]-electrochemical biosensors, by differential pulse voltammetry, electrochemical impedance spectroscopy and/or quartz crystal microbalance.

The changes in the dsDNA structural morphological conformation observed were related to the condensation and/or aggregation, distortion and/or unwinding of the double helix, binding of the molecules in the DNA grooves, and release of guanine and adenine residues. The drugs-dsDNA interaction mechanisms were proposed.

Acknowledgements: PhD Grant 232296/2014-6 (W.B.S. Machini) from CNPq/Brazil, projects FCT (UID/EMS/00285/2013) and Innovec'EAU (SOE1/P1/F0173).

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#### CAN ELECTROCHEMICAL BIO-SENSORS HELP FOOD TECHNOLOGISTS IN THE ASSESSMENT OF CEREAL AND PSEUDOCEREAL BIOACTIVE COMPOUNDS?

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The increasing interest of the relationship between health and nutrition has steered the scientific research towards the study of the role of specific food compounds (phytochemicals), which are naturally present in many foods, for the prevention of diseases.

The kernel of cereals and pseudocereals is a heterogeneous (composition and distribution of nutrients) and versatile system that adapts well to the technological evolutions addressed by the new nutritional, health and environmental needs as well as to the development of tailor-made and functional foods. Various bioactive compounds present in the cereal and pseudocereal kernel such as dietary fiber,  $\beta$ -glucan, arabinoxylans, fructans, phenolic compounds, alkylresorcinols, tocols, folates, resistant starch and others (De Paula et al., 2017a; De Paula et al., 2017b; Messia et al., 2019; Verardo et al., 2008; De Arcangelis et al., 2019; Panfili et al., 2008) can be successfully used as ingredients to produce tailor-made and functional foods. To evaluate the concentration of bioactive compounds in raw materials, and their fate during technological processes it is necessary to have reliable, sensitive, accurate and rapid analytical methods. Can electrochemical and/or optical biosensors help food technologists in the assessment of cereal and pseudocereal bioactive compounds? This point will be a matter of discussion during the presentation.

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#### OPTICAL SPECTROSCOPY COMBINED WITH VACUUM-FREE MATRIX ASSISTED LASER FRAGMENTATION AND TRANSFER (MALFT) FOR BIOSENSING

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Rapid detection and identification of bacteria with high sensitivity and selectivity is a challenging problem in clinical diagnosis, pharmaceutical, water distribution network, and food-processing technology.<sup>1</sup> The progress in optical and mass spectroscopy techniques has provided tools for identifying bacteria by spectroscopic fingerprinting. The matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectroscopy has recently gained on popularity in rapid identification of bacteria from infections as indicated by the worldwide introduction of this technique in hospitals.<sup>2</sup> Nevertheless, there are some important limitations associated with MALDI-TOF, such as i) limited sensitivity that requires a large amount of bacteria (at least 10<sup>5</sup> colony-forming units (CFU) per mL for each sample), and ii) although it is a viable technique for identifying unknown bacteria in pure form, its performance is restricted for mixed bacterial samples.

In this presentation, I will introduce an innovative approach taking advantage of MALDI matrices and Fourier transform infrared (FTIR) spectroscopy. The bacterial samples are fragmented and transferred to a carrier substrate by exposure to UV laser radiation. Figure 1 shows examples of FTIR spectra of *Bacillus subtilis* and *Escherichia coli* bacteria, pre-mixed with  $\alpha$ -Cyano-4-hydroxycinnamic acid (CHCA) matrix, and processed with a 248 nm laser delivering 10 and 30 pulses at 160 mJ/pulse. The results illustrate differences in absorption observed in the lipid and protein/polysaccharide bacterial regions. For instance, it can be seen that the IR lipid peaks, normally expected in the 2700 - 3000 cm<sup>-1</sup> region, did not appear for *B. subtilis*. The preliminary results suggest that the proposed technique could rapidly distinguish low concentrations of different bacteria and, potentially, different bacterial strains.



Figure 1. FTIR spectra of *Bacillus subtilis* and *Escherichia coli* bacteria, pre-mixed with CHCA matrix and processed by 248 nm laser, 160 mJ/pulse, for 30 pulses (red line) and 10 pulses (blue line), respectively, related to the lipid region (a) and the protein/polysaccharide region (b).

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# C-NANODOTS BASED LABEL-FREE DNA SENSING

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Here we report on the development of an assay for DNA sensing based on fluorescent carbon nanodots (CNDs). The developed assay even allows the rapid detection of gene mutations associated with human diseases, in particular, mutations in BRCA1, CFRT and MRP3 genes associated with breast cancer, cystic fibrosis and autoimmune hepatitis diseases, respectively. Blue fluorescent carbon nanodots were synthesized, from citric acid and ethylene diamine, and extensively characterized by different techniques (DLS, TEM, FTIR, XPS, UV-V and Fluorescence spectroscopy). Then their interactions with DNA were studied. The formation of a nanobioconjugate between C-nanodots and DNA was confirmed by DLS and fluorescence microscopy. Changes in the fluorescence intensity following interaction with single and double-stranded DNA (ssDNA and dsDNA) were used for a specific DNA sequence detection. The biosensor response was linear with respect to target DNA concentrations up to 200 nM with a detection limit of 270 pM. Moreover, the high selectivity allows not only to discriminate between wild type and mutated DNA samples, but also rapidly detect single gene mutations, without the need of labels.

Keywords: Assay, Cancer, Carbon nanodots, DNA recognition, Genetic diseases

#### ELECTROCATALYSIS AND PEROXIDASE-LIKE MIMICS OF POLYOXOMETALATES AND HIGHLY SENSITIVE ELECTROCHEMICAL DETECTION OF NITRITE, OF HYDROGEN PEROXIDE AND OF GLUCOSE

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Keggin type POMs (XM<sub>12</sub>O<sub>40</sub><sup> $n^-$ </sup>, X = P, Si/M = W, Mo, V). offer favorable accessibility for electron transfer from empty d orbitals for metal–oxygen  $\pi$  bonding. They can undergo a stepwise multielectron reversible redox process without any structural changes.

A new boron doped diamond (BDD) electrochemical microcells was modified, for rapid, selective and highly sensitive determination of nitrite, using a coating film of a monolacunary silicotungstate ( $\Box$ -SiW11O39<sup>8-</sup>) formed by cyclic voltammetry on the molecular p-phenylenediamine (PPD) electrodeposited on BDD. BDD/PPD/SiW11 electrodes showed excellent electrocatalytic activities towards nitrite ion. Under the selected conditions, the anodic peak maximum at -0.6 V was linear versus nitrite concentration in the 40  $\mu$ M - 4mM range, and the detection limit obtained was 20  $\mu$ M. The newly developed electrode has been successfully applied to the determination of nitrite content in real river water samples (1)

A new organic-inorganic material, based on an alpha-metatungstate  $[H_2W_{12}O_{40}]^{6-}$  cluster, was synthesized via the hydrothermal method by using aminopyridinium  $(APy)^+$  cations as the hybrid material, and characterized by FT-IR spectrometry and thermal analysis. A simple and reliable procedure was implemented for the synthesis of new composite materials obtained by the covalent grafting of  $(APy)_6[H_2W_{12}O_{40}]$  onto the surface of carboxylic acid functionalized single-walled carbon nanotube (SWCNT-COOH). The synergistic effect of single-walled carbon nanotubes on the electrochemical behavior of  $(APy)_6[H_2W_{12}O_{40}]$  was evidenced through the negative shift of the reduction peaks and their increased intensities - several times higher-which must be due to the energy level stabilization of  $(APy)_6[H_2W_{12}O_{40}]$  through electron transport from the LUMO orbitals of the SCWNTs to the LUMO orbitals of  $H_2W_{12}O_{40}$ , as shown on the Figure. The sensitivity of hydrogen peroxide detection was increased by a factor of 38.5 in presence of SWCNT, showing their high influence on peroxidase-like mimics of  $(APy)_6[H_2W_{12}O_{40}]$ .

Acknowledgements: The work was supported by Campus-France through PHC Maghreb # 39382RE

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#### LEAD (II) DETECTION IN WASTE WATER BASED ON ELECTROCHEMICAL BIOSENSORS FORMED WITH *DNAZYME* AND ACRIDINE-FERROCENE INTERCALATOR

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Lead ion  $(Pb^{2+})$  is a toxic metal pollutant, which is widely spread and plays significant roles in biological and environmental systems. Word Health Organization (WHO) gives provisional guideline value of  $10\mu g/L$  in tap water. So it's of great interest to develop simple, fast and reliable method for the sensitive and selective detection of  $Pb^{2+}$  to prevent lead exposure and poisoning.<sup>1</sup> Various sensors and bioreceptors have been designed to detect  $Pb^{2+}$ , DNAzyme based sensors have attracted much attention due to their advantages underlining their high selectivity and sensitivity.<sup>2</sup>

The introduction of nanomaterials in biosensor design has significantly contributed to advanced diagnostics and monitoring tools.<sup>3</sup> Herein, we described the design of DNAzyme based sensing platform, using hybrids nanomaterials MoS<sub>2</sub> nanosheets/Polypyrrole and DNAzyme/ redox intercalator to achieve efficient lead detection in waste water. A redox DNA intercalator was designed and synthetized based on acridine-ferrocene where acridine is selected for high affinity to bind double strand DNA and ferrocene as excellent redox marker.

A sequential modification strategy to elaborate the biosensor was performed based on electrochemical and chemical reaction. Firstly, the screen printed carbon electrode was modified through electrodeposition with MoS<sub>2</sub> nanosheets/Polypyrrole and then functionalized by functional group through amine electrooxydation. DNAzyme immobilization was performed through covalent attachment and Acridine-Ferrocene used as nucleobase binding and redox reporter was intercalated. We demonstrated that in the presence of Pb<sup>2+</sup>, the DNA-zyme is cleaved into two fragments and this dissociation leads to the release of the redox intercalator and the variation of current related to attached ferrocene.

Electrochemical studies showed that this biosensor is highly sensitive with a wide dynamic range of 1-200 ng.mL<sup>-1</sup> and a limit of detection of 1.3 ng.mL<sup>-1</sup>, which lower than recommended. Specificity was demonstrated by measuring various metal divalent ions thanks to selectivity of DNA-zyme. Furthermore, the biosensor enables analysis of lead trace in contaminated waste water such as river.



Acknowledgments: The authors' acknowledge project PHC N° 39382RE for supporting a part of this project.

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# SYNTHESIS TECHNIQUES OF MOLECULARLY IMPRINTED POLYMER: APPLICATION OF ULTRASOUND PROBE TECHNIQUE

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Molecularly imprinted polymers (MIPs) are synthetic materials that mimic the natural, biological antibody-antigen systems. They operate by a "lock and key" mechanism to selectively bind the target analyte with which they were templated during production [1]. There are multiple methods to prepare MIPs including (1) surface stamping or lithography, (2) phase inversion by mixing the polymer and the template to form their complex in a compatible solvent, then precipitating them by adding an incompatible solvent (3) synthesis of polymer from monomers in presence of the template. The last one is mostly used with different techniques discussed in this work. Among these synthesis techniques, thermal heating has been widely employed. However, it has some drawbacks such as the long synthesis time and relatively high temperature which is not convenient for some biological templates such as proteins and cells. Therefore, many alternative synthesis techniques have been proposed to overcome these drawbacks including photopolymerization, microwaveassisted synthesis, ultrasound-assisted synthesis and electro-polymerization. In this context, we focus our work on a rapid synthesis of MIPs decorated magnetic nanoparticles using highpower ultrasound probe. We succeed to decrease drastically the synthesis time from 24 h to few minutes [2,3]. Several parameters including synthesis time, applied amplitude and solvent were choice were studied. The synthesized materials characterized with Scanning/Transmission electron microscopy, Dynamic Light Scattering, Fourier Transform Infrared spectroscopy, X-Ray Diffraction and Vibrating Sample Magnetometer. We investigated and optimized various parameters to increase the binding capacity and imprinting factor of MIPs decorated magnetic nanoparticles. Under the optimal conditions, the resultant material has been successfully used as solid phase extraction coupled to colorimetric method for sulfonamide detection in spiked tap and mineral water.

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#### FLOWER-LIKE ORIGAMI PAPER-BASED ELECTROCHEMICAL DEVICE FOR THE DETECTION OF PESTICIDES

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Pesticides are largely used at worldwide level to improve the food production, fulfilling the needs of the global population, which is increasing year by year. Although persistent pesticides (e.g. DDT) have been replaced with less persistent ones, contamination of food, soil and water by pesticides remains an issue of public concern. To better manage this problem, EU sets regulations for a sustainable employment of pesticides by promoting the adoption of Integrated Pest Management (Directive 2009/128/EC). In this overall scenario, the detection of pesticides in liquid and aerosol solutions at low concentrations (ppb level) is required to accomplish the regulatory aspect and to preserve the health of environment and human being. Herein, we propose a flower-like origami paper-based device for the detection of several classes of pesticides by combining different enzyme-inhibition biosensors. This device was developed by integrating two different office paper-based screen-printed electrodes and multiple filter paper-based pads to load enzymes and enzymatic substrates. The versatile analysis of different pesticides was carried out by folding and unfolding the filter paper-based structure, without any addition of reagents and any sample treatment (i.e. dilution, filtration, pH adjustment) [1]. The paper-based platform was employed to detect paraoxon, 2,4-dichlorophenoxyacetic acid, atrazine and glyphosate at ppb level by exploiting the capability of these different types of pesticides to inhibit butyrylcholinesterase, alkaline phosphatase, tyrosinase, and peroxidase enzymes respectively. The degree of inhibition, correlated to the quantity of pesticides, was chronoamperometrically evaluated, monitoring the enzymatic activity in the absence and in the presence of pesticides by using a portable potentiostat. To improve the sensitivity, the paper-based electrodes were modified with carbon black nanoparticles in the case of platforms for 2,4-dichlorophenoxyacetic acid, atrazine and glyphosate detection or carbon black decorated with Prussian blue nanoparticles for the detection of paraoxon.

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# LARGE AREA ORDERED ARRAYS PLASMONIC NANOSTRUCTURES FOR HIGH-SENSITIVITY BIOSENSORS

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In this work, highly ordered array of plasmonic nanostructures have been fabricated developing a simple and reproducible approach based on nano-sphere lithography (NSL). This cost-effective method is based on the self-assembling of close-packed polystyrene (PS) particles at air/water interface, and enables the fabrication of colloidal mask characterized by a high quality crystal structure. Periodic array of metal nanostructures can be easily prepared on large-area by exploiting the interstitial geometry of this cheap lithographic mask. In particular, highly ordered array of gold nano-triangles distributed in hexagonal lattice, nanoholes and nanodisks have been fabricated on different kind of substrates. Optical biosensor based on Localized Surface Plasmon Resonance has been developed for the sensitive detection of Lipopolysaccharides (LPS). Gold nanostructures have been realized, used as optical transducers, functionalized with specific antibodies as sensing elements for the detection of LPS. After a proper functionalization step of the nanostructured transducers, Protein A was immobilized which contains an Fc antibody-binding specific domain allowing an oriented immobilization of antibodies.



Each functionalization step have been monitored by optical characterization by measuring the shift of the resonance peak. A good linear relationship between peak shifts and the LPS concentration has been demonstrated with a detection limit down to 10ng/ml. Further applications in quality control of pharmaceutical preparations and medical devices are in progress.

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#### BIOSENSOR PLATFORMS WITH MODIFIED ELECTRODES BASED ON ELECTROACTIVE POLYMERS PREPARED IN DEEP EUTECTIC SOLVENTS AND CARBON NANOTUBES

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Highly sensitive, reliable, reproducible electrochemical sensors and biosensors with low detection limits can be achieved by electrode surface modification with conductive nanomaterials and/or electroactive polymers [1]. For this purpose, we have been investigating using multiwalled carbon nanotubes (MWCNT), often decorated by gold nanoparticles, to increase the active surface area, with good electrocatalytic and synergistic effects. We have also used deep eutectic solvents (DES) as media for the preparation of electroactive polymer films as a way of tuning their morphological and structural properties, on carbon electrode or on MWCNT modified carbon electrode substrates. DES eutectic formation, done by direct mixing of two solid non-toxic components in the right proportions, occurs due to strong hydrogen bond interactions between a hydrogen bond acceptor, such as choline chloride, and a hydrogen bond donor such as urea, ethylene glycol or glycerol, that have amine or hydroxyl groups [3].

Electrodes modified with electroactive polymers, have been prepared by electropolymerisation in ethaline (choline chloride/urea) DES from appropriate monomers, together with MWCNT. Varying the synthesis experimental conditions enables tuning the nanostructure and surface morphology for electrochemical sensing applications, such as ascorbate oxidation, and improves the analytical parameters in relation to their analogues formed in aqueous solutions. The formation of the phenazine polymer poly(brilliant cresyl blue) and the triphenylmethane polymer poly(brilliant green) has been studied in detail using potentiodynamic and potentiostatic deposition in ethaline DES, and the influence of the addition of different anions in acid conditions to improve DES conductivity has been probed

The platform based on poly(brilliant cresyl blue)-DES/MWCNT has been used for immobilising glucose oxidase (GOx) and tyrosinase (Tyr) for glucose and catechol biosensors with improved analytical characteristics. The biosensor characteristics including enzyme kinetics, influence of pH, limit of detection and sensitivity have been evaluated and will be presented.

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#### TRANSITION METAL DICHALCOGENIDES BASED NANOHYBRID SENSORS AS USEFUL DEVICES IN FOOD POLYPHENOLS ANALYSIS

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Nanomaterials (NMs) have become elective analytical tools able to improve the performances and give rise to new opportunities, for both constructions of analytical devices and implementation of analytical methods. On the other hands, polyphenolic compounds (PCs) still continue to attract exceptional attention, for their well-known health benefits, for their technological role and also marketing [1]. In the last years, following the line traced by graphene, transition Metal Dichalcogenides (TMD) have emerged as electrode material for analytical purposes due to large available surface area and structural versatility

[2]. TMDs are compounds with a general structure  $MX_2$  where M is a transition metal and X is a chalcogen. These nanomaterials show unique electrical, optical, mechanical and catalytic properties along with large surface area. Despite, widely employed in energy conversion and storage, their properties have not yet been widely and deeply studied for (bio)sensing purposes. Herein, for the first time, the ability to detect polyphenols belonging to different chemical classes on an effective and regenerable carbon black/molybdenum disulfide nanohybrid screen-printed electrode (SPE-CB/MoS<sub>2</sub>) is demonstrated. The proposed SPE-CB/MoS<sub>2</sub> merges the ability of CB to improve the electrochemical response with the proprieties of MoS<sub>2</sub> to totally prevent polyphenols irreversible polymerization and adsorption onto the electrode surface occurring at classical 'carbon-based' electrodes. The proposed CB/MoS<sub>2</sub> sensor showed significantly improved analytical performances, noteworthy, the MoS<sub>2</sub> antifouling ability has been demonstrated for the first time using both PCS/flavonoids standards and complex samples thus proved to be an unexpected and useful characteristic. In brief, a regenerable and totally anti-fouling (classical catechins/polyphenols analysis drawback) screen-printed electrode (SPE) based on TMD for the cocoa (CO) catechins (CT) and olive oil PCs rapid quantification has been successfully realized [3,4]. Moreover, during this study, a new natural polyphenolic electrochemical mediator has been discovered. In particular, has been demonstrated the ability of cocoa powder (CO) and pure catechins (CT), to act as effective electrochemical mediators when electrodeposited onto carbon black (CB) modified screen-printed electrodes (SPE-CB). As proof of applicability, the SPE-CB-CT/CO has been employed for the detection of free (GSH) and total (GSH+GSSG) glutathione in blood samples. Definitely, this work contributes to further prove that nanomaterials (in this case TMDs and CB) are unique and useful analytical tools able to both tailor 'customize sensors' and giving rise to new analytical opportunities.

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# $\mathbb{OC}$ -21

#### **ONE-SIDED COVALENTLY ATTACHED ROBUST BILAYER (OSCAR-B) FOR** ELECTROCHEMICAL STUDIES OF BIOMOLECULAR INTERACTIONS

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Alzheimer's disease (AD) affects 46 million people today, and this number is expected to triple by  $2050^{[1]}$ . The main protein implicated in AD is amyloid- $\beta$ , a hydrophobic membrane protein that forms amyloidogenic plaques resulting in neural degeneration<sup>[2]</sup>. An emerging method of studying the aggregation properties of amyloid- $\beta$  is by electrochemistry, which provides a sensitive and versatile platform for studying biomolecular interactions. Traditionally, membrane proteins have been embedded in hybrid-lipid bilayer membranes (h-LBMs) that have been attached to a gold substrate through a thiol bond<sup>[3]</sup>. However, the main disadvantage of using this method is attributed to the reorganization of h-LBMs on gold substrate that might cause the detachment of thiol-Au bonds<sup>[4]</sup>. Herein, we developed an alternative model for h-LBMs by attaching a diazonium-based lipid onto the surface of a glassy carbon electrode (GCE), followed by the incorporation of a phospholipid that acts as the second layer. The construction of this covalently-immobilized h-LBM layer was verified using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) along with X-ray photoelectron spectroscopy and ellipsometry. Following this, amyloid- $\beta$ , was incorporated into the h-LBM and analyzed using CV and EIS. Our results show a progressive decrease in impedance as the incubation time of amyloid- $\beta$  increases, suggesting the formation of

nanopore complexes that might facilitate charge transport<sup>[5]</sup>.

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#### AN ORIGAMI PAPER-BASED LAB-ON-A-CHIP FOR PRECISION MEDICINE IN ALZHEIMER DISEASE

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In the last decade, an increase of higher mortality rates and healthcare costs for treatment, hospitalization and care assistance for Alzheimer and Parkinson diseases has been reported, with an estimated cost of approximately € 130 billion/year [1]. The recent trend of precision medicine has boosted the personalization of medical care in several fields including neurological diseases, thanks to recent diagnostic and therapeutic tools discovered and developed, respectively [2]. In this overall scenario, we propose a novel paper-based lab-on-achip to deliver a cost-effective and easy to use sensing tool for a customised administration of drugs in Alzheimer disease. Among several drugs, we have designed the device for evaluating the efficiency of alkaloid compounds (e.g. Physostigmine) which can inhibit in a reversible way the cholinesterase enzyme. Since the activity of cholinesterase is different among the patients, the administration of the customised amount of drug can improve the treatment and the quality of patient life, avoiding side effects due to the overdosage. In detail, we exploited office paper to print the electrode and VividTM Plasma Separation membrane to threat the blood sample as well as to load the reagents needed for the measurement, delivering a reagent free analytical tool. The calibration curve of BChE obtained in real blood sample gave a linearity between 2 and 12 U/mL with a sensitivity of 0.050  $\pm$  0.004  $\mu$ A mL/U. The Physostigmine inhibition activity against cholinesterase enzyme was measured in untreated blood matrix, in the range between 0.01 and 1 µM. A linearity up to 0.5 µM was obtained, characterised by the following equation  $y = (9 \pm 2) + (111.4 \pm 7.1) x$  with a  $R^2 = 0.9605$  and a LOD (calculated as the amount of drug which gives 10% of inhibition) =  $0.01 \,\mu$ M.

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#### ONE-STEP REDUCED AND FUNCTIONALIZED GRAPHENE OXIDE BASED BIOSENSOR

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Graphene is attracting much attention, because of its unique physical and chemical properties and its potential applications. Currently, graphene has mainly been produced by micromechanical exfoliation, epitaxial growth or chemical vapour deposition and by reduction of exfoliated graphite oxide (GO) [1]. The functionalization of graphene with specific functional groups it is a great deal of interest since it allows not only its solubilisation in aqueous media but also endow it with specific properties, which open new potential applications of this nanomaterial. Hence, molecules carrying quinones moieties that can simultaneously reduce GO and modify it by not covalent bonding, allowing to keep its properties and endowing it with new functionalities, would have great applications. In this work we describe a one-step method of reducing exfoliated graphene oxide (GO) to produce dispersions of functionalized reduced graphene oxide (rGO) using N,N-bis(3,4-dihydroxybenzylidene)-1,2-diaminobenzene (3,4-DHS). This compound is a polyhydroxylated Schiff base ligand which contains two hydroquinone groups and an aromatic ring that can interact non-covalently with the graphene sp2 network. 3,4DHS acts simultaneously as reductant and functionalizing agent [2], without any other additional reagent. The resulting hybrid nanomaterial (rGO-DHS) incorporates quinone moieties by a noncovalent functionalization, just by conjugation with the planar structure of the graphene, avoiding destruction of the  $\pi$ -conjugated skeleton and loss of the electronic properties of graphene. In addition, the electrochemical properties of the reversible redox system guinone/hydroguinone are incorporated to the graphene, obtaining a nanomaterial with synergistic properties. Once the novel hybrid nanomaterial was characterized by several techniques, including Raman spectroscopy, Fourier transform infrared (FTIR), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), confirming the presence of the characteristic quinone/hydroquinone moieties, it has been used to construct a reagent-less lactate biosensor based on the functionalized graphene and lactate oxidase. The hybrid nanomaterial acts as an acceptor of the electrons generated in the enzymatic reaction giving a biocatalytic current proportional to the concentration of L-lactate. The biosensor applicability has been demonstrated by the direct determination of the L-lactate content in white wine without any treatment of the sample.

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Acknowledgments: The authors are grateful to the Ministerio de Economía, Industria y Competitividad (project No. CTQ2014-53334-C2-1-R and CTQ2015-71955-REDT) and Comunidad de Madrid (S2013/MIT3029) for the financial support.

### GLUCOSE BIOSENSOR BASED ON NANOTRUCTURED SAMARIUM OXIDE

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Glucose biosensors have been the subject of a great number of published papers since 1962 where Lyons and Clark published the first device of this kind for glucose determination. There is a great interest in glucose monitoring by easy and cheap methods for diverse fields especially in biomedical devices for diabetic patients. In this work it will be presented a glucose biosensor based on Sm<sub>2</sub>O<sub>3</sub> deposited by single bath electrochemical deposition. Surface morphology and composition was characterized by X-ray diffraction, X-ray photoelectron spectroscopy and scanning electron microscopy. Electrochemical characterizations were carried out by cyclic voltammetry, electrochemical impedance spectroscopy, and fixed potential amperometry. The operational conditions of GOx/Sm<sub>2</sub>O<sub>3</sub>/Au/SiO<sub>2</sub>/Si electrode were optimized for glucose determination in standard solutions, and all analytical parameters were calculated. Finally, glucose was determined in blood serum samples at -0.3 V (vs. Ag/AgCl) with values in accordance with those found in literature

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#### Acknowledgements

Financial support from the Romanian Ministry of Research and Innovation through Operational Program Competitiveness 2014-2020, Project: NANOBIOSURF SMIS 103528

# PAMAM-BASED PLATFORMS FOR THE ELECTROCHEMICAL BIOSENSING OF $\frac{\text{MYCOTOXINS}}{\text{Sondes BEN AISSA}^{1,2}}, \text{ Gaëlle CATANANTE}^1, \text{ Noureddine RAOUAFI*}^2 \text{ and Jean-Louis}$

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The performance of a biosensor is critically dependent on the biofunctionalization step of the sensor platform. Accordingly, the right immobilization protocol of the capture probe and linkage play a pivotal role in the detection effectiveness. Among countless options, supramolecular dendritic architectures have shown immense potential in the design of sensing platforms. The interesting properties of dendrimers, such as their structural uniformity, globular shape, monodispersity, high functional group density, hydrophilicity, nanometric size and the existence of dendritic crevices, make them very good candidates for immobilizing biomolecular species as bioreceptors. For instance, polyamidoamine (PAMAM) dendrimers can be exploited while developing highly sensitive biosensors, in particular aptasensors. In this context, our work focuses on the modification of disposable screen-printed carbon electrodes (SPCEs) with hyperbranched third generation (G3) PAMAM molecules. This study highlights the electrodeposition feasibility of PAMAM on carbon surface under mild conditions, mainly using cyclic voltammetry. We used the as-modified electrode to develop a new E-platform for the sensitive detection of Aflatoxin B1 (AFB1) using specific anti-AFB1 aptamers tagged with methylene blue as a redox probe. Voltamperometric readout using Differential Pulse voltammetry (DPV) as well as Square Wave Voltammetry (SWV) showed promising results with an enhanced sensitivity, good stability and greater accessibility of the probe for the targeted mycotoxin. This platform lends itself as a versatile immobilization layer for any type of bioreceptor in the development of electrochemical biosensors.



Schematic representation of Aflatoxin B1 aptasensor based on PAMAM-modified screen-printed carbon electrode

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#### AN ELECTROCHEMICAL BIOSENSOR EXPLOITING ALGAE-BASED CARBON BLACK MODIFIED SCREEN-PRINTED ELECTRODES DEVELOPED WITHIN THE BILATERAL AGREEMENT ITALY-MOROCCO ALGAE-CB

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This work describes the outcomes resulting from the Bilateral Agreement ALGAE-CB based on the scientific/technological cooperation between Italy and Morocco. ALGAE-CB vision is to provide drinking water access to a widen number of people in EU and non-EU Mediterranean countries, to ensure geographic, socio-cultural, and economic equalities. In Mediterranean region, water is a scarce and fragile resource due to rapid population growth, socio-economic development, mass tourism, agriculture, being responsible of spreading tons of pesticide. Among pesticides, atrazine continuous to be found as pervasive pollutant of watersheds and drinking water systems, despite banned in European Union and linked to harm to wildlife and humans. As a consequence, several regulations have been stated to set the maximum level of atrazine concentration in different matrices, as drinking water and inland surface waters (EU Dir. 2013/39/EC); however, there is still an urgent requirement for its monitoring in different ecosystems. ALGAE-CB intends to fill the strong gap in monitoring environmental pollution emerging from information from European Commission. To this aim, a novel amperometric biosensor was designed exploiting the alga Chlamydomonas reinhardtii immobilised on carbon black (CB) modified screen-printed electrodes (SPEs). The biosensor was able to detect atrazine in the nanomolar range both in standard solutions and surface water samples (from Sebou River). A linear response was obtained by serial additions of atrazine in the concentration range 0.1-6.6 M allowing the construction of a calibration curve using the linear regression described by the equation y = 0.98 ( $\pm 0.013$ ) - 0.083 ( $\pm 0.0054$ ) x, with an R<sup>2</sup> = 0.9874. A detection limit of 1.7 nM was obtained for I<sub>20</sub> value (calculated as  $2.6 \times \sigma \times I_{20}/100 - 2.6 \times \sigma$ ) and 4.3 nM for I<sub>50</sub> value (calculated as  $2.6 \times \sigma \times I_{50}/100 - 2.6 \times \sigma$ ). The biosensor showed satisfactory working (10 hours) and storage (2 weeks) stability and negligible interference from toxic compounds at safety limits (EU Directive 2008/105/EC for inland surface water) as cadmium, lead, arsenic, copper, paraoxon, bisphenol A, slight matrix effect and satisfactory recovery values.

Keywords: Chlamydomonas reinhardtii, carbon black, screen-printed electrodes, atrazine

#### Acknowledgement

This work was supported by ALGAE-CB Bilateral Project Italy-Morocco 2018/2019 within the Joint Research Projects Programme of the Italian National Research Council.

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#### **ORTHOGONAL ANTIBODY-REGULATED DNA NANOSTRUCTURE ASSEMBLY** AND DISASSEMBLY

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Here we report a rational strategy to orthogonally control assembly and disassembly of DNAbased nanostructures using specific IgG antibodies as molecular inputs. [1] To do this, we first demonstrate that the binding of a specific antibody to a pair of antigen-conjugated split inputstrands induces their co-localization and reconstitution into a functional unit that is able to initiate a toehold strand displacement reaction. [2] The effect is rapid and specific and this approach results versatile and in principle, generalizable to any antibody for which an antigen can be attached to a DNA anchoring strand. In support of this claim, we have demonstrated that two different DNA circuits can be controlled in an orthogonal way in the same solution by using two different IgG antibodies as Inputs. Such antibody-regulated DNA-based circuit has been then employed to control the self-assembly of two distinct DNA tubular structures by using DNA circuits controlled by two different IgG antibodies in the same solution in an orthogonal way. Similarly, we can autonomously control in the same solution [3] the assembly of a DNA nanostructure with a specific antibody and trigger the disassembly of the same structure with another antibody. Given the growing importance of antibodies as molecular markers in diagnostics and as therapeutic drugs, the antibody-controlled DNA nanostructures we report here may prove of utility in a range of applications, including controlled drug-release, point-of-care diagnostics and in-vivo imaging.



Figure 1. Antibody-controlled DNA circuit re-engineered to trigger the assembly of DNA nanotubes.

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#### RECENT ADVANCES IN SIGNAL AMPLIFICATION STRATEGY BASED ON OLIGONUCLEOTIDES, ENZYMES AND NANOMATERIALS FOR MICRORNAS DETECTION

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Nucleic acid-based electrochemical biosensors are considered to be effective, simple, robust and inexpensive analytical tools for the early diagnosis of cancer biomarkers. Recent studies demonstrate that the aberrant expression of a new class of RNA named microRNAs correlates with the initiation and progression of a variety of cancers.

Early diagnosis of cancer becomes a crucial concern to achieve a curative treatment. For that, in order to improve the sensitivity of nucleic acid-based electrochemical biosensors, several amplification strategies have recently been developed. These strategies are classified according to the use of nanomaterials, enzymes or oligonucleotides. Nanomaterials have the particularity of increasing the conductivity, specific surface area and grafting density of biomolecules. Enzymes have a significant rate of renewal, which generates a significant amount of electroactive product. On the other hand, oligonucleotides will promote the weaving of a large support for the binding of electro-active molecules responsible for amplifying the detection signal.

Herein we present several approaches dedicated to microRNAs biomarker detection. These approaches aimed for the developed of new electrochemical based on the use the new amplification strategies.

The advantages of these different amplification strategies will be presented. However, challenges remain to improving the stability of the bio-platform, improving the sensitivity of the biosensor and reducing the analysis time, which are critical parameters for on-site detection.

**Keywords:** Nucleic acid biosensor, Amplification strategy, Nanomaterials, Enzymes, Oligonucleotides, MicroRNAs cancer biomarkers.

# Posters

#### ELECTROCHEMICAL DETECTION AND IDENTIFICATION OF FUNGAL SPORES

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Fungi inhabit almost all kinds of environments and can live on many substrata containing organic matter [1]. Unfortunately, a lot of them cause various damages e.g.: human and animal diseases, allergies, infections of plants, food moulding, spoiling of buildings. Moulds produce huge number of spores that spread in the earth atmosphere unreservedly carrying a large amount of mycotoxins, their second-order metabolites. Some mycotoxins are harmful and have cytotoxic, mutagenic, neurotoxic and even carcinogenic effects on human and animal organisms [2]. A crucial factor in a battle against fungi is their early and effective detection and identification. It is a challenge because most of applied methods are based on discrete microscopic detection, observations and culturing that are time consuming processes.

The goal of our work is to construct a new label-free electrochemical sensor to detect and identify fungi. Fungi samples taken from four locations in south Finland: *Penicillum bialowiezense*, *Cladosporium limoniforme*, *Mucor circinelloides*, *Trichoderma viride*, *Alternaria alternata*, *Aspergillus jensenii*, *Stachybotrys chartarum* and *Chrysonilla crassa* were the objects of our studies. Suspensions of spores of mentioned fungi were prepared in two buffers (pH 4.75 and pH 8.00) and their electrochemical properties were characterized using screen-printed three-electrode systems by a broad spectrum of voltammetric techniques.

Our results show that all fungi samples contain various electroactive substances, which are oxidized in the potential range from 0.1 to 1.2 V. The number and positions of the observed signals depend strictly on fungus species and pH of suspension. We suppose that registered signals result from the mycotoxins produced by fungi [3]. We have identified one signal common for most fungi but simultaneously, we have described sets of signals characteristic for individual species and different from each other.

Financial support from Business Finland (former Finnish Funding Agency for Innovation-TEKES), Project No. 554/31/2016, is acknowledged.

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#### ELECTROCHEMICAL DETERMINATION OF GLUTATHIONE, PHYTOCHELATIN AND PHYTOCHELATIN SYNTHASE ACTIVITY

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Introduction. The synthesis of nanoparticles, especially silver (AgNPs), for various biomedicinal applications is a topical issue<sup>1</sup>. Free SH groups provide reducing properties, allowing binding of toxic compounds<sup>2,3</sup>. Glutathione (GSH) and phytochelatins (PCs) occurs in different species of animals, plants, and bacteria<sup>4,5</sup>. The study was aimed at monitoring the change in the level of thiol compounds in plants exposed to AgNPs. Experimental part. The absorbance spectra were recorded by a UV–VIS spectrophotometer. Determination of thiols by DPV was performed at Autolab. The chromatographic system coupled on-line to the VIS detector was used. The PCs activity was determined by Nakazawa's study<sup>6</sup>. Results and discussion. In experimental work, we focused on green synthesis, which uses the reduction potential of plant extracts to form AgNPs. Compounds present in plant extracts catalyze the formation of nanoparticles and modify their surface. Electrochemical methods have been employed in the analysis of GSH and PCs and also in the study of oxidation and reduction of the sulphur group in cysteine residues (a reduced -SH and oxidized -S-S- forms). When the oxidation of reduced forms of the peptides (GSH and PCs) was started at -0.8 V, two reversible anodic peaks at -0.45 and -0.30 V were obtained. In addition, the levels of thiol compounds in plants exposed to AgNPs were studied. The enzyme PCs synthase is activated by the presence of metal ions. GSH, PCs and PCs synthase levels varied depending on the applied concentration and exposure time. Conclusion. The introduction of the method for evaluating phytochelatin formation and the phytochelatin synthase catalytic activity will bring new insights into the evaluation of the AgNPs toxicity to cell cultures<sup>7</sup>.

#### Acknowledgments

The work was carried out with the support of the H2020 CA COST Action CA15114, INTER-COST LTC18002.

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#### SET-UP OF A DIRECT COMPETITIVE ELIMC ASSAY FOR A RAPID AND SENSITIVE DETECTION OF MICROCYSTINS

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Freshwater poisoning caused by cyanobacteria is nowadays a worldwide serious problem. In fact massive cyanobacterial proliferations, when dominated by toxic strains, can be a source of potent neurotoxins and hepatotoxins, responsible for gastroenteritis and liver damages in humans. Among cyanotoxins, microcystins (MCs) are a hazardous class of hepatotoxins (more than 100 different MC congeners) produced by cyanobacteria such as Microcystis, Oscillatoria, Anabaena and Nostoc usually found in lakes, water reservoirs and recreational facilities. MCs are also a drinking water public health issue with a provisional drinking water guideline of 1  $\mu$ g/l for MC-LR (WHO, 1998). Because of a widely conservative approach to health protection, the value of 1 µg/l should be referred to the sum of different MCs concentrations, considered as equivalent of MC-LR. The management of surface and drinking water is essential to protect both human and fauna health, and the development of cheap, reliable, and non-time consuming analytical methods to detect MCs is becoming very important. In this work, a competitive ELIMC (Enzyme-Linked Immuno-Magnetic Colorimetric) assay for the detection of MCs is presented. The assay was designed by combining the advantages of magnetic beads (MBs) with the high performing and stable reagent solutions supplied by a commercial ELISA kit. Our strategy is based on a single step competition procedure in which pre-coated and blocked immuno-magnetic particles (MBs) are incubated for a short time with all the required reagents (toxin, toxin-HRP and specific antibodies). A colorimetric readout was performed by adding a TMB substrate solution. A working range between 0.15 and 5 ppb was obtained for MC-LR. Thanks to the use of MBs, Systea is working to transfer and integrate the manual assay in an automated analyzer for a realtime monitoring of MCs in fresh and drinking water.

### A « LAB-ON-A-DISC », LOD, FOR AQUATIC ECOSYSTEMS MONITORING

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Natural and drinking water monitoring requires a real-time detection in situ of different kinds of pollutants before they cause any damage on aquatic ecosystems. In this purpose we are developing an early warning system based on algae metabolism measurements.

The device is a Lab-On-a Disc that integrates a network of sensors and unicellular algal

biosensors. It consists in disks stacking, each fulfilling a specific function. A network of organic light-emitting diodes (OLEDs) is located on the upper disk, these OLEDs emitting in different spectral ranges.

Microfluidic chips are arranged on the second disk (d2). Different species of algae and samples studied are inserted inside the chips. Electrochemical and physical sensors are also on d2 for physico-chemical measurements as pH, O2, nitrates, conductivity.



Organic photodetectors (OPDs) on the two lowest disks permit the measurement of chlorophyll fluorescence of algae due to OLED excitation. In the same way, the activity of enzymes located on external membrane of algae can be assessed by using fluorescent substrate. These measurements provide informations on algal metabolism which can be disrupted in presence of different pollutants family.

Thus, this tool permits to carry out different analyses simultaneously on different algal species. It therefore appears to be a promising early warning system for water managers.

#### VOLTAMMETRIC SENSOR BASED ON ELECTRODEPOSITED MOLECULARLY IMPRINTED CHITOSAN FILM ON *BDD* ELECTRODES FOR CATECHOL DETECTION.

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This work is focused on the development of a simple, effective, sensitive and biocompatible molecular imprinted sensor for the electrochemical recognition of catechol. Polymeric chitosan matrix has been electrodeposited by chronoamperometry in the presence of catechol followed by elution with 0.1 M KCl on a boron doped diamond (BDD) (1). The electrochemical response of the sensor analyzed by cyclic voltammetry (CV) indicates that the sensor shows an excellent reproducibility and repeatability to catechol detection in the range of 0 to 25  $\mu$ M, with a detection limit of 5.7  $\cdot 10^{-7}$  M and high selectivity to catechol recognition versus different phenolic compounds. The results obtained in a red wine denotes the extraordinary capability to detect catechol in a complex matrix.

#### Acknowledgements:

C. Salvo Comino thanks the Universidad de Valladolid for MINECO grant.

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#### COPPER COATED WITH CARBON NANOFIBER/PDMS COMPOSITE AS ANODES IN MICROBIAL FUEL CELLS

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Microbial fuel cells (MFCs) have been recognized as a potentially sustainable biotechnology that involves the direct conversion of organic matter from wastewater into electricity using bacterial biofilms as biocatalysts<sup>1,2</sup>. Electroactive bacteria (EAB) use organic matter as electron donors and are capable of extracellular electron transfer to the anode. However, the electricity production of MFCs remains too low for their large-scale applications in wastewater treatment. These restrictions are mainly due to the slow kinetics of electron transfer from bacteria to electrode surfaces at anode electrode<sup>3</sup>. The development of low cost and high performance anodes, is one of the key factors for practical implementation of MFCs. Modification of electrode surfaces is a promising strategy to improve MFC performance<sup>4</sup>. Here, we report a new functionalization process to improve interfacial electron transfer, biocompatibility and corrosion resistance of copper electrodes used as anodes in MFCs. Copper electrodes prepared by surface modification with thin layer (500  $\mu$ m) of conducting composite made of polydimethylsiloxane (PDMS) doped with commercially available carbon nanofibers (CNF), have been used as anodes in MFCs. Electrochemical characterization showed that the corrosion rate of copper electrode in an acid solution decreased after CNF-PDMS coating. Electric characterization demonstrated that the maximum power density generated by MFCs after 14 days with Cu/CNF-PDMS anodes (70 mW.m-2) is 3 times higher and more stable than that with unmodified copper. The cyclic voltammetry analysis indicated that the electrochemical activity of the modified anode was enhanced significantly after 14 days and the electron transfer was facilitated by CNF-PDMS modification. Microscopic observations and electrochemical characterization showed that CNF-PDMS composite improved biocompatibility and corrosion resistance of the copper anode surfaces. These results confirmed that the CNF-PDMS modification is a promising approach to improve the properties of anode materials for MFC application.

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#### HEAVY METAL SENSING VIA NOVEL MAGNETIC BEADS FUNCTIONALISED WITH NUCLEIC BASES DERIVATIVES

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Heavy metal pollutants have aroused a widespread concern owing to their adverse impact on the human health and the environment even at very low concentrations. They are considered as persistent contaminants since they are non-biodegradable and can accumulate all along the food chain. Their onsite monitoring in water is still a challenge to overcome in order to reduce health risk factors. Nucleic bases have recently shown increasing interest in the chemical, biological and therapeutic fields. In addition, their complexing power with heavy metals makes them good ligand

candidates. Thus, our work focuses on the design of a novel sensor for heavy metal ion detection using magnetic beads functionalized with nucleic bases derivatives. The magnetite nanoparticles were prepared by chemical co-precipitation method. Their size was measured by dynamic light scattering. They were subsequently coated with 3-aminopropyltriethoxysilane and melamine followed by the attachment of the synthesized nucleobases hydrazide derivatives that were characterized by the Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance and mass spectrometry. The overall steps involved in the functionalization process were confirmed by FTIR. Square wave voltammetry measurements conducted in citrate buffer pH 4 showed a difference in the peak current before and after incubation with Pb (II) indicating that these beads can be used to capture and preconcentrate heavy metals improving therefore their electrochemical detection.

#### TOWARDS THE DEVELOPMENT OF AN IMMUNO-RT-PCR FOR DETECTION AND QUANTIFICATION OF S. AUREUS ENTEROTOXIN B

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Bacterial protein toxins, such as *Staphylococcus aureus* enterotoxins (SEs), play an important role in food safety as they cause serious food-related illnesses worldwide. Health effects caused by enterotoxins can be observed already at very low doses. The most common SEs are SEA and SEB. SEB is not only involved in food poisoning but identified as potential biological weapon of war and terrorism. The reported detection limits of available commercial systems for the detection of SEs range from 0.5 to 2 ng enterotoxin per g of food. Nonetheless these methods require improvement as it is reported that the established intoxication dose could be underestimated due to the constraining detection limits.

The aim of this work is the development of an immuno-RT-PCR for an ultra-sensitive detection and quantification of SEB. To achieve this goal an "in-house" spectrophotometric ELISA sandwich, using the same couple of antibodies and the same format architecture, was set-up. This format involves the use of an immobilized polyclonal capture antibody and a detection antibody conjugated with biotin for the next interaction with streptavidin, which in turn binds more biotin-HRP molecules for a colorimetric read out. Antibody, streptavidin and biotin-HRP concentrations were optimized. A strategy to minimize non-specific adsorption of the bioreagents, on the ELISA plate, was adopted. Under these conditions the enzymatic assay was able to detect 0.5 ng/mL of SEB. The same colorimetric sandwich assay was then repeated by substituting the conventional ELISA plate (not suitable for real-time PCR instrument) with RoboStrips specially designed for immuno-PCR technique, obtaining similar results. After that, a biotinylated single strain DNA, to replace streptavidin-HRP, was designed and purchased together with the forward and reverse primers. The amplification of this DNA sequence, *via* RT-PCR using SYBR Green as florescent marker, should significantly increase the sensitivity of the method.

#### Acknowledgements

The authors wish to thank the national project, "Nuove strategie analitiche per la valutazione del rischio dei prodotti lattiero-caseari: sviluppo di metodi Real time PCR e immuno-Real time PCR per la determinazione del *Bacillus cereus* e sue tossine" (IZS ME 10/15 RC), Ministry of Health for financial support.

#### A COMPREHENSIVE PICTURE OF GLYCAN EPITOPE COMPOSITION OF A CELL WALL BIOMASS SAMPLE, VIA GRAVIMETRIC AND ELECTROCHEMICAL SENSING

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A plant fiber is a composite-like natural material, where the reinforcing fiber is cellulose, the matrix is lignin, and hemicelluloses is the interface that makes the two previous constituents compatible. The rigidity and strength of the composite are ensured by the cohesion between the fiber and the matrix. Physical properties of wood are therefore polysaccharides dependent. Fine analyses of lignocellulosic biomass will contribute, among other things, to enhance the understanding of the difference in defense mechanisms, according to the wood essences. Besides, a better knowledge of all chemical wood components, that vary widely, even for the same species, will constitute an undeniable asset for any industrial application involving wood. This work concerns the design and realization of electrochemical and gravimetric "wood based" immunosensors for label-free and follow-up of specific antibody/antigen pairs interactions. The investigated transduction techniques, gravimetry (via the use of surface acoustic wave sensors) and electrochemistry have already revealed their great potential and complementarity for chemical and biological species detection [1-3].

We aim to overcome the main limitations encountered in the major classical methods needed for wood constituent's quantification.

Preliminary results will be presented and discussed for both biosensors in terms of sensitivity and selectivity and will be compared to those obtained from Enzyme-Linked Immunosorbent Assays (ELISA) technique.

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#### ELECTROCHEMICAL ASSAY FOR SENSITIVE DETECTION OF NUCLEIC ACID OF AFRICAN SWINE FEVER VIRUS

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Introduction. African Swine Fever Virus (ASFV) is a DNA virus of the Asfivirus genus of the Asfarviridae family that is found in blood, body fluids, and internal organs<sup>1,2</sup>. ASFV was described more than 40 years ago<sup>3,4</sup>. This virus spreads pandemically and the mortality rate of the virus-related disease ranges from 90 to 100  $\%^{5,6}$ . The aim of this study was to propose the detection of specific nucleic acid (NA) of ASFV using electrochemical hybridization biosensor. Experimental part. The absorbance spectra of nanoparticles were measured in a UV-VIS spectrophotometer. Determination of DNA and/or RNA by DPV and/or SWV was performed using Autolab. The parameters of the measurement were as follows: initial potential 0 V, end potential -1.8 V, accumulation time 120 s, step potential 5 mV, modulation amplitude 25 mV, frequency 200–500 Hz, the volume of injected sample 10 µL. Results and discussion. Electrochemical methods are suitable techniques for the study of ODNs, PCR products and their components. The AdTS detection limit of NA samples (signals of adenine and cytosine around -1.3 V) was about hundreds of attomoles per 5 µL drop. To increase the selectivity and sensitivity of NA determination, we have prepared electrochemical labels (CdTe) by green synthesis<sup>7</sup>. Using these labels, specific probes for the target DNA were labelled. A meat product (ham) was used as a sample. After DNA extraction, PCR reaction to the target sequence was performed. The PCR product was subsequently recognized by the probe labelled with CdTe. The proposed procedure has increased the sensitivity of PCR product detection by 85 %. Conclusion. We have designed a sensitive electrochemical method for detecting viral nucleotide sequence. The detection hybridization probe used was labelled with green synthesis-prepared CdTe.

#### Acknowledgments

This work was supported by the grant projects AMOR No. QK1920113 from the Ministry of Agriculture of the Czech Republic and CA LTC18002. References

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#### SIMULATION/EXPERIMENT CONFRONTATION, AN EFFICIENT APPROACH FOR SENSITIVE SAW SENSORS DESIGN

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Chem/biosensors in general, and surface acoustic wave (SAW) sensors in particular, are promising analytical tools for analytes detection, in gas and liquid media, as they offer several advantages such as low cost, high sensitivity, selectivity, low limit of detection, and the possibility of real-time monitoring.

The current evolution of environmental and public health standards has led researchers to direct their research towards the development of increasingly sensitive sensors, which should be, in addition, miniaturized and wireless. Several strategies have thus emerged to increase SAW sensors sensitivities, mainly the increase of the operating frequencies (up to some GHz) or the confinement of the energy nearby the surface, by depositing suitable materials (on the SAWs sensing area), which act as guiding layers. We have chosen the second route to convert our Shear Horizontal SAW (SH-SAW) delay-lines sensors to Love wave mode ones. Finite Element Method (FEM) simulation become therefore essential for any optimization step preceding their realization, in a clean room, and further investigation for analytes detection [1, 2].

In this work, Finite Element Method (FEM) simulation, thanks to COMSOL Multiphysics software, was first used to optimize the thickness of the Love wave layer, which permits infine to enhance SAW sensors sensitivity. Here, SU-8, an epoxy-based resin, has been chosen for its interesting physical properties, and for its potential to be spin-coated as homogeneous films with thicknesses ranging from few hundred nanometers to some micrometers. Comparison between 2D and 3D simulations indicates that all pertinent parameters were not altered, whether we use one or the other method. As results were comparable, we opted for the 2D simulations as they allow a substantial time saving, especially since the structure dimensions used in modeling are the same as that of the real SAW sensor (few millimeters). Based on preliminary results, the optimized structures will be realized and tested to confront experiment's findings to calculation predictions.

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#### DESIGN OF A LEAD SENSOR BASED ON GALACTOMANNAN EXTRACTED FROM CAROB

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In this work, a receiver layer of an electrochemical sensor for the detection of some heavy metals is made from a polysaccharides kind extracted from carob seeds called galactomannans. These extracts are characterized by different physicochemical methods to understand their structures and to detect their different functional groups. To understand the phenomena occurring at the interface, the detection of metal ions in liquid media is characterized electrochemically using cyclic voltammetry (CV) and square wave voltammetry (SWV). The results obtained reveal a very high sensitivity with a small limit of detection to lead ions over a linearity wide range between  $10^{-10}$  ppm and  $10^{-2}$  ppm.

Keywords : Galactomannan, polysaccharide, sensor, SWV, CV.

# **P-13**

#### USE OF POLYSACCHARIDES EXTRACTED FROM THE "OPUNTIA FICUS INDICA" PRICKLY PEAR RACKETS TO CAPTURE CADMIUM IONS IN HYDRIC ENVIRONMENTS

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The present study aims at optimizing the extraction conditions and at establishing the physicochemical properties of the different polysaccharide fractions of "Opuntia Ficus Indica" prickly pear snowshoes. The resulting extracts contain a variety of biologically active molecules and are used for the design of an electrochemical sensor for the detection of heavy metals in aquatic environments. The performance of this (bio) sensor is established by two electrochemical methods namely cyclic voltammetry and square wave voltammetry. This biosensor has a better sensitivity towards cadmium Cd<sup>+ 2</sup> ions. This is well illustrated in the calibration curves with a detection limit of the order of  $10^{-12}$  M and a good stability in a wide range of linearity ranging from  $10^{-7}$  M to  $10^{-3}$ M.

Keywords: Prickly pear, Opuntia Ficus Indica, Polysaccharides, Biosensor, Heavy metals, Cyclic voltammetry, Square wave voltammetry.

#### DEVELOPMENT OF IMMUNOSENSOR BASED ON GOLD INTERDIGITATED MICROELECTRODES FOR MEASUREMENTS OF HEART FAILURE BIOMARKERS IN SALIVA

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Cardiovascular diseases (CVDs) are disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. CVD is a major cause of human death in both developing and developed countries. According to the World Health Organization (WHO), 17.9 million people die each year from CVDs, an estimated 31% of all deaths worldwide

According to the WHO, the diagnosis of CVD has been based on 3 criteria, which are characteristic chest pain, diagnostic electrocardiogram (ECG) changes, and elevation of the biochemical markers in their biofluidic samples. Although, ECG is expensive, time-consuming and give a poor diagnostic test for CVD, because about half of the CVD patients who present to the Emergency Department show normal or no diagnostic electrocardiograms, which makes early diagnosis of CVD more difficult [1]. Hence the importance of developing new rapid and low-cost biosensing devices for the detection of biochemical markers that are related to CVDs-

TNF- $\Box$  is one of these biomarkers-.

Chemical and electrochemical surface modification are the best strategies to ensure the covalent binding of biological molecule. Thiols on gold are the most used. However, this method doesn't allow micro-adressing of the molecules of interest.

In this work TNF- was quantified by competitive detection procedure with Ag-TNF- on electrode's surface and Ag-TNF- in solution toward polyclonal Ab-TNF- First, gold interdigitated working electrodes were functionalized by electrodeposition of 4-aminobenzaldehyde by cyclic voltammetry. Second Ag-TNF- was covalently bonded to the electrode through the reaction between primary amines and aldehydes.

The Ag-TNF- was quantified by a competitive detection through making a mixture between fixed concentration of Ab-TNF- and decreasing concentrations of Ag-TNF- from 60pg.mL<sup>-1</sup> to 5pg.mL<sup>-1</sup>. The measurements were carried out by using electrochemical impedance spectroscopy (EIS).

**Key words:** Interdigitated gold electrode; TNF-, competitive detection; electrochemical impedance spectroscopy (EIS).

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#### Acknowledgments :

The authors acknowledge the financial support from the EU H2020 research and innovation program entitled KardiaTool with grant agreement N° 768686 and from CAMPUS FRANCE program under grant agreement PHC PROCOPE 40544QH and from PHC Maghreb N° 39382RE and from CNRST.

#### ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY DETERMINATION OF GLYPHOSATE USING A MOLECULARLY IMPRINTED CHITOSAN

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Glyphosate (GLY) is a widely used herbicide for agriculture around the world, it can be found in soil, water, plants, animals, and human inhabited areas with potential toxicological and environmental damage [1, 2]. Hence, its detection is garnering more interest. In this study, a simple electrochemical sensor for GLY detection in the water based on molecular imprinted chitosan film (MIPs) was developed for the first time. Meanwhile, the fabricated sensor showed high sensitivity and good selectivity toward to GLY in a linear range from 0.31 pg/ml to 1  $\mu$ g/ml.

The sensor was constructed by electrodeposition of chitosan (CS) and GLY mixture on the gold microelectrode surface, the modified electrode was immersed in solution of sulphuric acid for crosslinking. Finally, MIPs/Au was obtained after removing GLY from the film with methanol and acetic acid solution. A non-imprinted polymer sensor (NIPs) was prepared similarly, except that the template molecule was absent in the electrodeposition step. Then the microelectrodes were characterized by Electrochemical Impedance Spectroscopy (EIS) in  $[Fe(CN)6]^{-3/-4}$  with phosphate buffer saline (PBS) solution before and after incubation.

The electrodeposition step changes the microelectrode's surface as seen by its aspect under the microscope and by the charge transfer resistance (R) increase. A gradual increase of the impedance was observed with the increase in GLY concentration (y=0.087x+0.673). In order to assess the effectiveness of the imprinting, the experiment was repeated using NIPs/Au (y=0.006x+0.078). Moreover, the sensor selectivity was performed using water containing gluphosinate-ammonium (GLU) (y=0.011x+0.093), then chlorpyrifos (CHL) (y=0.002x+0.07). An electrochemical sensor for detection of glyphosate was developed by creating an imprinted polymer layer of CS on a gold microelectrode. The preliminary results show a high sensitivity, low detection limit and good selectivity.

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#### Acknowledgments

The authors acknowledge the financial support from the EU H2020 research and innovation program entitled KardiaTool with grant agreement N° 768686 and from CAMPUS FRANCE program under grant agreement PHC PROCOPE N° 40544QH and PHC Maghreb N° 39382RE.

#### GLYCOLIPID-SENSITIZED PLATFORM FOR SPECIFIC MONITORING OF ANTIBODIES

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Many proteins from the plasma membrane of eukaryotic cells are tethered using a glycolipid attached to the C-terminus of the proteins, which complex is named glycosylphosphatidylinositol anchored protein (GPI-APs) [1]. GPI-APs play a crucial role in cell signaling and cell adhesion and have implications in health and diseases. GPI-APs on the cell membrane and GPIs without attachment to a protein (free GPIs) are found in abundance on the surface of several protozoan parasites, such as *Trypanosoma brucei*, *Plasmodium falciparum and Toxoplasma gondi*i [2]. Recently, the detection of anti-glycan IgM or IgG antibodies has been used also to detect and differentiate between acute and latent toxoplasmosis patients as well as between colorectal cancer patients and healthy individuals [3] [4]. Detection of IgG, IgM and IgA antibodies is commonly based on serological assays [5]. These methods have some limitations such as poor efficiency, cross-reactivity and need for sophisticated laboratory equipment and qualified personnel. In this context, the aim of this work is to develop

a fast, highly sensitive and specific label-free electrochemical biosensor for monitoring antiglycan IgG and IgM antibodies in sera from toxoplasmosis seropositive patients. The biosensor uses a synthetic GPI glycolipid as bioreceptor, which is immobilized through a linear alkane thiol phosphodiester on screen-printed gold electrodes. The antigen-antibody interaction was detected and quantified by electrochemical impedance spectroscopy (EIS). The resultant GPI-modified platform allowed us to estimate the concentration of toxoplasma antibodies in serum ranging from 250 to 1000 UI. This proof-of-concept holds great potential for the discrimination and differentiation of a variety of medical conditions, including colorectal cancer.

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# EFFECT OF THE DILUTION GAS ON THE OPTICAL AND STRUCTURAL PROPERTIES OF NANOCRYSTALLINE SILICON DEPOSITED BY PECVD

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Recently, nanocrystalline silicon (nc:Si) films have attracted great interest because of his more interesting properties such as a wide bandgap, high electrical conductivity, high charge carrier mobility, high doping efficiency, high optical absorption coefficient and great stability against light soaking comparing to amorphous layers. In plasma-enhancedchemical vapor deposition (PECVD) the film structure depends strongly on the processing parameters such as substrate temperature, gas precursors, gas dilution, gas mixture, power density.... In this study we focus on the effect of the dilution gas (H<sub>2</sub> or He) on the optical and structural properties of thin silicon films grown by PECVD.



We found that the refractive index (Fig.1) of the layers using hydrogen as dilution gas is slightly greater than that of the layers produced using helium. This behavior is predictable since hydrogen plays the role of catalyst that promotes the dissociation of silane molecules.

The bandgap value Eg (Fig.2) deduced for the sample using H<sub>2</sub> as dilution gas is typical of nc-Si layer (Eg>1.8 eV) whereas for the sample deposited using He, Eg value is typical of a hydrogenated amorphous silicon layer (1.7 <Eg< 1.8 eV). The X-ray diffractogram of the sample deposited using H<sub>2</sub> as gas dilution shows a beginning of crystallization which is manifested by a peak which appears at  $2\theta = 28^{\circ}$  attributed to the orientation (111) of the crystalline silicon.

#### BIOSENSOR BASED ON FLOW-MODE MICROBIAL FUEL CELL FOR WASTEWATER QUALITY MONITORING AND EARLY WARNING SYSTEMS: COPPER IONS AS TARGET ANALYTE

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The qualities of municipal and industrial wastewater vary over time and have a direct impact on the performance of wastewater treatment plants (WWTPs). The presence of a high concentration of toxins, including toxic metals, could cause serious interruptions or irreversible damage of WWTPs. It is primordial to monitor in real time the presence of toxicity in wastewater. However, most of chemical tests for wastewater quality monitoring are done off-site and cause the long time delay for WWTPs to recover after shocks by toxicity. Online monitoring or online early warning systems of wastewater toxicity plays an important role in providing safe wastewater and for timely precaution and solution in order to ensure a proper functioning of WWTPs. Copper ions has been selected as model analyte, since they cause adverse effects on microorganisms or substructures (cells) and/or disturb their activity. Therefore, they cause toxicity to the environment and human health. In this work, a new biosensor based on flow-mode Microbial fuel cell (MFC) has been developed for the detection of copper ions in wastewater in real time. MFCs sensor are devices based on the degradation of organic matter by electrogenic microorganisms naturally present in wastewater for electricity generation. The presence of copper ions affects the electrochemical activity of microorganisms leading to a decrease or increase in the MFCs' electricity generation. The output voltage or current of an MFC was depending on the analyte concentration. Mature electroactive biofilms were formed on anodes after 15 days with a characteristic peak at around -0.2 V vs. Ag / AgCl. Three modes were used for the formation of biofilms (RE330 Ohm, 0.5 V floating potential, 0.1 V (amperometry mode) .The structuring of biofilms through an external resistor of 330 Ohm between anode and cathode showed the best performances in terms of current density (located between 2200 and 5000 mA.m<sup>-3</sup>) and electrical power output (located between 500 and 800 mW.m<sup>-3</sup>). Several parameters affecting the analytical performances of MFCs such as the effect of flow rate, medium nature, the presence and the absence of copper ions during biofilm formation and matter transfer mode were investigated. The optimal MFCs were then tested for the detection of copper ions in a concentration range below the value set by WHO (2 mg / L).

**Key words:** Wastewater treatment plants, Microbial Fuel cell, Biosensor, Toxic metals, Flow mode, Cu (II), Early warning systems.

#### Acknowledgements:

Authors greatly acknowledge PHC Maghreb 2019 project N°41382WC for its support to the realization of this work