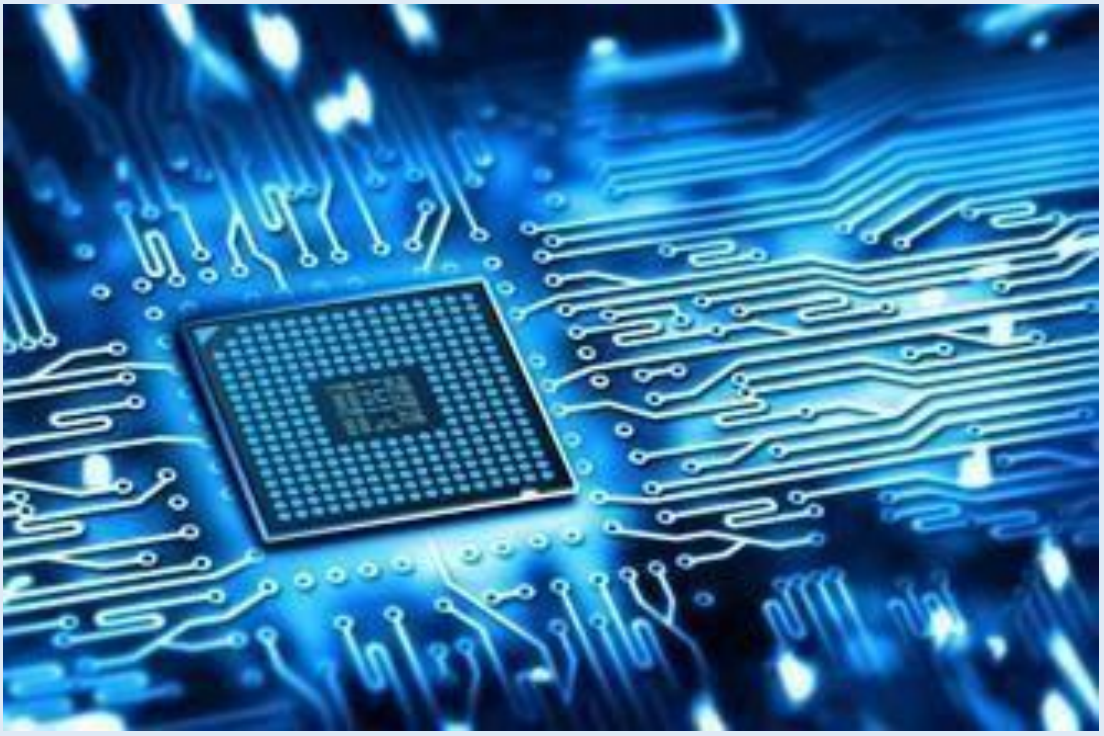
ABSTRACTS OF THE XXV TRANSFRONTIER MEETING ON SENSORS AND BIOSENSORS

It reaches the 25th edition of the Transfrontier Meeting on Sensors and Biosensors and will be held online for the first time.



IRTA

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APTAMERS AND MIPS

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Development of real time detection and quantification tools for bacterial spores responsible of food spoilage – SPORES QUANTUM

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Over the past ten years, consumer concerns on health, nutritional and organoleptic qualities of sterilized foods have been constantly increasing. In response to this demand, canning manufacturers are constantly improving the packaging of their products and the process. These modifications may favour the emergence of certain biological contaminants because of their adaptation to their environment but also because of their resistance to the treatments used during the transformation and/or manufacturing process. The economic losses in the canning industries caused by the spoilage of appertized foodstuffs at 55°C (hygiene indicator) are attributable in 70% of cases to the presence of spores of non-pathogenic, but highly resistant sporulated microbial species: *Geobacillus stearothermophilus* or *Moorella thermoacetica*. Food products regularly contaminated by this bacterial species are dairy products (skimmed milk, condensed milk, milk powder), appertized vegetables such as peas and green beans or ready meals. In order to limit economic losses and food waste, a quantification of the risk of *G. stearothermophilus* and *M. thermoacetica* can be carried out before thermal treatment, but aerobic and anaerobic cultivation methods are long and tedious and do not allow a result to be obtained in less than 3 to 5 days. All the existing standardised and alternative methods suffer from the complexity of their implementation and the time required to obtain results.

The SPORES-QUANTUM project, supported by the Carnot Qualiment Institute, proposes to develop two aptasensors dedicated to the detection and quantification in real time of *G.stearothermophilus* and *M. thermoacetica* spores. This project aims designing biosensor tools for each bacterial spore using as biorecognition element a DNA aptamer obtained by SELEX technology. To date, the first step of selection of specific aptamers from *G. stearothermophilus* spores has been completed. The oligonucleotides selected during the SELEX protocol have been sequenced by high throughput sequencing (HTS) and a bioinformatics analysis has highlighted some over-represented sequences that are potential candidates for target recognition. These aptamers are currently being sorted to determine the most sensitive/specific candidates. Once selected, the resulting aptamers will be integrated into optical and electrochemical biosensors.

Gold nanoparticles aptamer assay for Nandrolone detection

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Nandrolone is an androgenic anabolic steroid (AAS) functioning as a growth promoting agent which helps to gain muscle weight. Its AAS properties have let to its exploitation as a doping agent in sports and horse racing, whereas it is also used as an animal feed additive [1]. However, adverse side effects are associated with nandrolone accumulation in the body such as endocrine, cardiovascular, skin and psychiatric disorders [2]. Nowadays, liquid or gas chromatography-mass spectroscopy is routinely used for laboratory-based analysis of nandrolone [3]. Aptamers are attractive biorecognition molecules with great potential in analytical applications [4]. They are artificial synthetic nucleic acids (RNA/DNA) that bind specifically to their target and are selected through an *in vitro* iterative process called Systematic Evolution of Ligands by Exponential enrichment (SELEX) [5]. In this work, nandrolone aptamers were identified using a classical SELEX process with nandrolone-Sepharose resin in combination with high-throughput Next Generation Sequencing. The binding properties of the selected aptamers were characterized and finally, gold nanoparticles were employed in combination with the aptamers for assay development. The colorimetric assay is based on the red-to-blue color change of gold nanoparticles after nandrolone binding, and it enables the rapid screening of samples suspected of containing nandrolone.

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Biomimetic MIP-based Electronic Tongue for biogenic amine sensing

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Biogenic amines (BAs) are a family of chemical species that can be found in food and beverage originated from the microbiological decarboxylation of amino acids. BAs can be used as a freshness and proper handling indicator in different scenarios such as food and beverage preservation control, hygienic control in industries or as a quality parameter during transportation and/or storage. Furthermore, their presence can be an indicator of food or beverage spoilage; thus, it might be considered not suitable for consumption. Although, there are several studies that relate its consumption to different disorders and disease such as gastric and intestinal problems, allergic responses, headaches, nausea and asthma, not all countries have legislation that regulate the maximum levels of BAs present in foodstuff and beverages.

Because of the aforementioned, there is a need to control and prevent its proliferation in degradable products. In this work, an array of different voltammetric sensors is presented to determine and quantify mixtures of different BAs. The sensor array is built from graphite-epoxy composites (GECs) that incorporate a biomimetic artificial receptor that selectively binds and captures the different BAs, i.e., histamine, tyramine and tryptamine. This artificial receptor is a polymeric material named molecularly imprinted polymers (MIP) [1]. This artificial receptor polymers are designed and synthesised towards a specific template molecule (Fig.1), which is indeed our analyte and object of study, in this case the different BAs.

In order to demonstrate the selectivity and specificity of the built sensors, several studies were performed to characterize its morphology as well as its electrochemical performance. Next, an electronic tongue was developed to achieve the identification and quantification different BAs mixture; an approach that would not be possible without the use of such advanced chemometric tools due to the overlapping voltammetric signals.

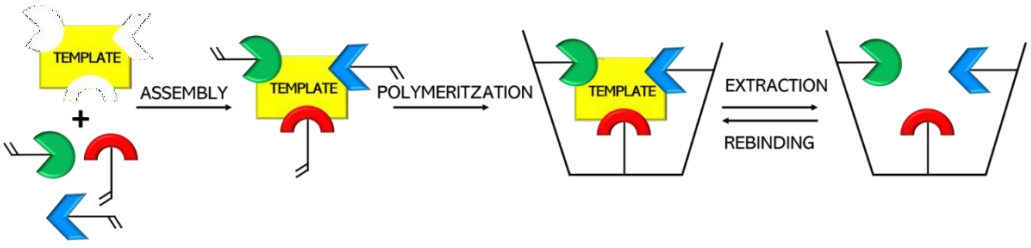

Fig 1. Schematic representation of MIP synthesis.

[1] A. Herrera-Chacón, X. Cetó, M. del Valle, Molecularly imprinted polymers - towards electrochemical sensors and electronic tongues, Anal. Bioanal. Chem. (2021). https://doi.org/10.1007/s00216-021-03313-8.

Electrosynthesis of MIP for the Detection of Kynurenic Acid for ACLF

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Decompensation of liver cirrhosis, and its further progression to ACLF, causes 1.2 million deaths per year [1]. The deregulation of the intestinal microbiota triggers the development of cirrhosis, i.e., altering the gut microbiome composition would have an inherent effect on the concentration of several metabolites, including the kynurenic acid, one of the byproducts of the tryptophan degradation via the kynurenine pathway (KP), which has caught the attention for being a potential target [2]. Furthermore, as it is detectable in the bloodstream and other accessible fluids, an overexpression of KA could indicate a possible alteration in the microbiota [3].

This project proposes the scalable electrochemical fabrication of a selective, chemically inert, and stable molecularly imprinted polymer (MIP) for the detection of a KA, using o-phenylenediamine as the monomer. The utmost important challenge that needs to be addressed in the first stages of developing a MIP would be to study the binding interactions among the monomers and between the monomer and the template under a porogenic solvent, which must be guaranteed to form a spontaneous and stable template-monomer complexation. Currently, we use electrochemical and spectroscopic techniques to characterize our system and corroborate the step-by-step optimization, considering the ionic form of KA in several matrices. Unlike current and typical immunoaffinity-based approaches, MIPs have demonstrated a higher sample load capacity for small molecules (with MW 3 kDa), resulting in higher recoveries for further analytical applications, as well as displaying a slightly higher selectivity and specificity towards smaller targets [4,5], bypassing the need for expensive equipment and time-consuming protocols.

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MATERIALS

- **Franc Paré** (UAB), "Single-Walled Carbon Nanotube-Based Sensor for Direct Amperometric H_2S Detection"

- **Alexandre Moreno Diaz** (CNM), "Fibroin Functionalization of ISFETs for the Production of Long-Life (Bio)Sensors"

- **Maria A. Tapia** (UB), "Bismuthene-Based Sensors for the Voltammetric Determination of Metal Ions"

- **Laura A. Uribe** (URV), "Cyclodextrins as Supramolecular Antidotes: Inclusion Complexes of Veratridine Neurotoxin with Different Cyclodextrins Enhance Cell Viability in Neuroblastoma 2-a Cells"

Single-Walled Carbon Nanotube based sensor for direct amperometric H_2S detection

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In recent years, a great emphasis has been put in repurposing waste chemicals into renewable feedstocks [1]. Sulfur compounds, those that conform the sulfur cycle, are among some of the most important ones. Specially hydrogen sulfide (H_2S), which is a water-soluble toxic gas, severely hazardous even at low concentrations for prolonged exposure, which can be found in wastewaters or as a product of sulfate leftovers treatment. In response, new bio-scrubber designs for sulfide oxidation are being developed to deal with it [2]. Nevertheless, these systems require constant monitoring, for which it is necessary to come up with adequate analytical systems.

Amperometric sensors are capable of in-situ measurements, a crucial requirement for constant monitoring of bioreactors, due to their fast response and general lack of sample pretreatment. Moreover, inkjet printing technology allows for the fast, reproducible, economic, and versatile fabrication of miniaturized electrodes [3]. Tuned with the appropriate 2D materials transductors, said modified electrodes can easily become suitable sensors for prolonged track of analytes.

Here, a miniaturized inkjet-printed Au electrode (1 mm^2) modified with Single-Walled Carbon Nanotubes (SWCNTs) and Poly(VinylAlcohol) (PVA) sensor is presented. SWCNTs allow for a lower oxidation potential and protect the sensor from sulfur poisoning, while PVA increases the overall mechanical stability of the modified sensor and an allows for a longer linear range.

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Fibroin functionalization of ISFETs for the production of long-life (bio)sensors

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Two main drawbacks are now limiting the long-term implementation of biosensors, namely the ageing/poisoning of the sensor and the stability of biological recognition element. Silk fibroin from the silkworm *Bombyx mori* is now considered in the production of biosensors for being able to retain biomacromolecules, e.g. enzymes or antibodies, for one year and without compromising their integrity or function.[1] Apart from molecular retention, the nanoporous nature of the silk-fibroin matrix also allows the filtration of macromolecules from the medium, minimizing the transport of macromolecules to the transducer surface and the well-known "matrix effects".
In this work, the previous properties of silk fibroin films have been exploited in the production of long-life ISFETs (from Ion Sensitive Field Effect Transistor)[2] for their future implementation in cell culture platforms.

Silk fibroin film on the ISFET gate were produced after drop-casting the water-based silk fibroin solution (5% w/w) on the silicon nitride device, drying (18h) and vacuum annealing (18h) at room temperature and physiological pH (pH = 7). The annealing resulted in the crystallization of silk-fibroin, the formation of thin fibroin films (20-30 µm) and the nanoporous matrix. Due to its high transparency and small thickness, silk films do not absorb in the visible light (Fig. 1), although IR confirms its presence by the presence of characteristic peaks corresponding to the amide groups from the beta-barrels.

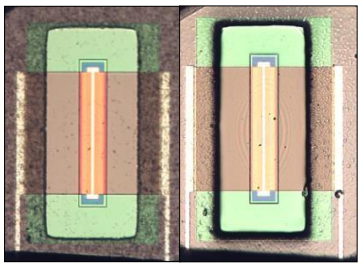

Figure 2: ISFET gate area (left). Fibroin modified (right).

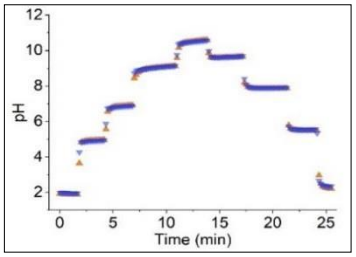

Figure 2: Dynamic recording during pH measurements with ISFET (orange) and a modified ISFET (blue).

The functionalized ISFETs were characterized and compared to non-functionalized counterparts by measuring pH changes in buffer solutions, cell culture medium and diluted blood samples. Measurements from the ISFETs were compared to those provided by commercial pH-meters (CRISON MicropH 2002) and a gasometer, which are used here as gold standard techniques. The response time, sensitivity, repeatability and stability of the transducers were evaluated.
In buffer solutions, functionalized and non-functionalized ISFETs presented similar sensitivities, although the response time was slightly larger by the former due to the presence of the membrane. However, functionalized ISFETs presented higher stabilities and reproducibility when in contact with complex biological matrices such as culture medium or blood, where non-functionalized ISFETs presented higher drifts associated to matrix effects and the poisoning of the transducer surface.
Future steps are the implementation of biomacromolecules, e.g. ionophores, in the silk fibroin film for monitoring ions in culture media.

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Bismuthene- Based Sensors for the Voltammetric Determination of Metal Ions

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2D layered nanostructures have arisen a great interest in the last decade due to the excellent features of these materials, which make them excellent candidates for electrochemical sensing, among others. After the great success of graphene, the 2D layered materials based on pnictogen elements (i.e. elements of group VA) such as phosphorene, bismuthene, antimonene, and arsenene, have gained significant impact in the last years. In particular, the pnictogen bismuth crystallizes with a layered rhombohedral structure and can subsequently be exfoliated by top-down methods [1]. Thus, the use of exfoliated layered bismuth (bismuthene) for the modification of voltammetric sensors could be a much better alternative for the detection of metal ions to the most conventional bismuth-based electrodes.

In this work, a bismuthene carbon-based screen-printed electrode (2D Bi_{exf}-SPCE) was developed by drop-casting an exfoliated bismuth suspension on the working electrode surface of a SPCE. 2D Bi_{exf}-SPCE was analytically examined for the simultaneous voltammetric determination of Cd(II) and Pb(II) as model metal ions. Moreover, 2D Bi_{exf}-SPCE was compared not only with bare SPCE but also with other bismuth-based screen-printed sensors i.e., bismuth nanoparticle-modified SPCE (BiNP-SPCE) and sputtered bismuth SPE (Bi_{sp} SPE). Out of all the tested bismuth-based sensors, 2D Bi_{exf}-SPCE demonstrated the best analytical performance, providing, for a 120 s preconcentration time, a linear response from 0.2 to 25.0 µg L^{-1} for both Pb(II) and Cd(II) and LODs of 0.06 and 0.07 µg L^{-1} for Pb(II) and Cd(II), respectively [2]. Finally, the developed 2D Bi_{exf}-SPCE demonstrated the capability of detecting Pb(II) and Cd(II) ions in a certified estuarine water reference material with very good trueness and remarkable reproducibility.

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Cyclodextrins as supramolecular antidotes: inclusion complexes of veratridine neurotoxin with different cyclodextrins enhance cell viability in neuroblastoma 2-a cells

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Abstract: Cyclodextrins (CDs) are cyclic oligosaccharides formed by 6, 7 or 8 glucopyranose units (α, β or γ cyclodextrin) (***Fig 1***) bonded together by ether bonds and forming a cone-like shape with a very hydrophilic exterior and an apolar central cavity. The main interest in cyclodextrins lies in their ability to form supramolecular host-guest complexes with several compounds. Given their low toxicity, great biocompatibility, and good complexation capacity with a variety of molecules, cyclodextrins have been extensively used as host molecules in the pharmaceutical, cosmetic, agrochemical, food, and biomedical industries [1]. On the other hand, veratridine (VTD) (***Fig 2***) is a neurotoxin that acts by blocking the voltage-gated sodium channels (VGSC) of cell membranes [2]. Symptoms of VTD intoxication include intense nausea, hypotension, arrythmia, and loss of consciousness. The treatment for the intoxication is mainly focused in treating the symptoms, meaning there is no specific antidote against VTD [3]. With this aim, we were interested in studying the molecular interactions of VTD with CDs. Since VTD is a lipid-soluble alkaloid, we hypothesized that it could form stable inclusion complexes (IC) with different types of CDs, resulting in changes of its physicochemical properties. We studied the formation of the IC of VTD with β-CD, γ-CD and SBβCD by UV-Vis, and NMR spectroscopy. Finally, with an interest in understanding the effects of the VTD-CDs molecular interactions, we performed cell-based assays (CBAs) on neuro 2-a cells. Our findings reveal that the use of different amounts of CDs have an antidote-like effect on the cells, significantly increasing cell viability and thus opening opportunities for novel research on applications of CDs and VTD.

Keywords: cyclodextrins; veratridine; neurotoxin; inclusion complex, neuroblastoma 2-a, cell-based assay

Figure 1. Cyclodextrins molecules

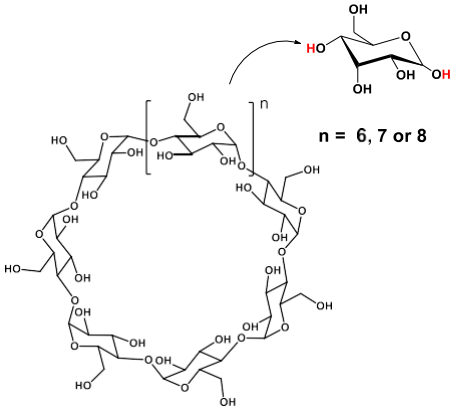
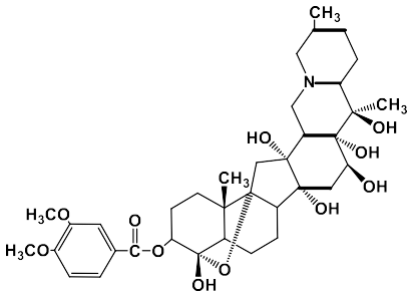


Fig 2. Veratridine molecule



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FABRICATION TECHNIQUES

- **María Jesús Ortiz-Aguayo** (UAB), "Thyroid Hormones Determination by Inkjet Printing Sensing Platforms Based on Graphene Hybrid Nanobiomaterials"

- **Massimo Urban** (ICN), "Challenges and Opportunities of Inkjet Printing with a Consumer Setup for Biosensing Application"

Thyroid Hormones Determination by Inkjet Printing Sensing Platforms Based On Graphene Hybrid Nanobiomaterials

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Thyroxine (T4) is an important biological hormone which can be considered as an iodoamino acid derivative of thyronine, produced in the thyroid gland. Thyrotropin (TSH) is a pituitary hormone that plays a decisive role in the control of thyroid gland. The determination of both thyroid hormones has practical clinical significance for the diagnosis of hyperthyroidism and hypothyroidism diseases. Considering this aspect, a wide range of analytical methods for the detection of the analytes, including immunoassay, chemiluminescence, mass spectroscopy and high-performance liquid chromatography, among others, have been developed [1]. This type of analysis provides feasible results. Nevertheless, requires qualified staff, special facilities and is time consuming.

For this reason, the aim of this project is to develop an electrochemical device manufactured by Inkjet Printing technology for free detection of T4 and TSH. For the manufacture of our electrochemical device, two main aspects are considered: The use of materials that amplify the electrical signal and find supramolecular scaffold that possess specificity towards the target analyte [2]. For this task, printed devices are modified with a hybrid – nanomaterial, consisting of reduced graphene oxide (rGO) tuned with Au nanoparticles (Au-NPs) and a biorecognition agent [3], different thiolate cyclodextrins (x-CD). Recognition of the analytes is accomplished via supramolecular chemistry, due to the formation of an inclusion complex between cyclodextrin with T4 and TSH. Morphological and electrochemical characterization of the final device was carried out to ensure the proper workability of the electrode, showing a sterling response and proper sensibility and limit of detection (LOD).

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Challenges and opportunities of inkjet printing with a consumer setup for biosensing application

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Inkjet printing as a fabrication technique for electronic devices has been known since few decades[1]. Recently, efforts have been put to use this technique for the production of nanobiosensing platforms, considering the needs and necessities of the field[2]. One of the possible approaches for it is to use consumer office-like equipment for the fabrication of these nanobiosensing devices[3]. The benefits of this system are in the ease of use and in the simple operational steps. The fabrication does not require trained personal, and large amounts of devices are produced in short times. Advances in nanomaterials broaden the combinations of functional nanoinks for the devices. The limitations are mainly related to the difficult access to all the printing parameters, thus the main challenge becomes to adapt the designs and materials to the printers. The possible outcomes are exciting, and this approach can lead to an easy production of devices for medical applications and everyday life.

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MAGNETIC BEADS

- **Melania Mesas** (UAB), "Magneto-Actuated Immunoassay and Magneto-Actuated Electrochemical Immunosensor for Enhancing the Detection of *Legionella pneumophila* Retained in Filters

- **Greta Gaiani** (IRTA), "Biosensor for the Detection of the Ciguatoxin-Producing Species *Gambierdiscus australes* and *Gambierdiscus excentricus*"

Magneto-actuated immunoassay and magneto-actuated electrochemical immunosensor for enhancing the detection of *Legionella pneumophila* retained in filters

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Communicable diseases are responsible for hundreds of thousands of deaths and an enormous burden of morbidity worldwide. The accurate identification of pathogens in the samples remains a major issue to disease control, since the burden of infectious disease would reduce if appropriate diagnostic tests were more widely available in the developing world as well as in low-resource settings[1, 2]. New technology and diagnostic-tests that are needed includes solid-phase separation techniques, since diagnostic targets can be thus concentrated increasing the sensitivity, for instance magnetic particles and other specialized materials, which are able to bind to the targets of interest and concentrate them before testing.

Accordingly, a magneto-actuated immunoassay and a magneto-actuated electrochemical immunosensor for the detection of *Legionella pneumophila* in contaminated water samples and filters was developed. A preconcentration method that combines filtration with the actuation of modified magnetic particles was designed, allowing the separation and preconcentration of the pathogen from high-volume samples. Furthermore, the evaluation of the specificity of the method was tested by analyzing cultures of *E. coli* and *L. pneumophila* separately and in combination.

The development of the electrochemical immunosensor for the detection of legionella allows the improvement of the limit of detection (LOD) and to overcome some of the bottlenecks related with the environmental bacteria, because of their low concentration in high-volume samples and the time consuming procedures usually performed for their detection, such as microbiological culture that requires several days[3].

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Biosensor for the detection of the ciguatoxin-producing species *Gambierdiscus australes* and *Gambierdiscus excentricus*

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Dinoflagellates of the genera *Gambierdiscus* and *Fukuyoa* are known to produce several bioactive compounds, including the potent neurotoxic ciguatoxins (CTXs), which are able to accumulate in fish and through the food web. When fish contaminated with CTXs is ingested by humans, it can result in an intoxication named ciguatera. Within the two genera of dinoflagellates, only some species are able to produce toxins, and *G. australes* and *G. excentricus* have been highlighted to be the most abundant and toxic. Although the genera *Gambierdiscus* and *Fukuyoa* are endemic to tropical areas, their presence in subtropical and temperate regions has been recently recorded. In this work, three primers modified with oligonucleotide tails have been designed within the D1-D3 region of the 28 S LSU ribosomal DNA. Two primers are species specific for *G. australes* and *G. excentricus* and the third one is in common, and they have been exploited in a multiplex PCR system to get amplified products of both species at the same time. Then, species-specific capture probes have been immobilized on magnetic beads (MBs) and exposed to PCR-amplified products. Each species-specific MB-capture probe complex has been immobilized on a working electrode, using a magnet underneath of a dual screen-printed carbon electrode array, and amperometric signals have been recorded after exposure to TMB Enhanced One Component HRP Membrane Substrate. With this configuration it was possible to achieve a limit of detection of 10 cells for both *G. australes* and *G. excentricus.* The development of this biosensor allowed the simultaneous detection of *G. australes* and *G. excentricus* species in field samples. This is a rapid and cost-effective strategy for detection of two toxic species, which will certainly contribute to ciguatera risk assessment, guaranteeing seafood safety.

TRANSDUCTION STRATEGIES

- **Chloé Aymard** (UPVD), “Development of a New Electrochemical Immunosensor for a Rapid and Sensitive Detection of Enrofloxacin in Meat Samples”

- **Zaida Herrero-Medina** (URV), “Strategies to Increase the Efficiency of Photosynthetic Microorganism-Based Biophotovoltaic Cells”

- **Mounira Alkassar** (IRTA), “Electrochemical Detection of Viable Neuroblastoma (Neuro-2a) Cells Using a Tetrazolium Salt”

- **Cansu Pinar Yenice** (URV), “Carborane- or Metallacarborane-Linked Nucleotides for Redox Labeling. Orthogonal Multipotential Coding of all Four DNA Bases for Electrochemical Analysis and Sequencing”

- **Aude Gandar** (UPVD), “Spectroelectrochemical Quantification of Sunscreens”

Development of a new electrochemical immunosensor for a rapid and sensitive detection of enrofloxacin in meat samples

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According to the World Health Organization, antimicrobial resistance is one of the main health threats that humanity will have to confront over the next decades. The control of the use of these medicines in veterinary field is one the promising strategies in order to reduce the risk of their presence in the food chain.

In this context, the main objective of TESTACOS project is the development of new tools to control antibiotic residues – especially fluoroquinolones (FQ) – in live animals as well as in meat commercialized for retail sale.

For this purpose, a new electrochemical immunosensor was developed to detect FQ in meat samples. This biosensor is based on the immobilization of anti-quinolone antibodies onto screen-printed dual carbon electrodes. Simultaneously, a new electrochemical probe was synthesized by conjugating difloxacin and aminoferrocene, whose oxidation was measured at +0.2 V vs. Ag/AgCl by differential pulse voltammetry. The detection principle was based on the competitive binding of this conjugate and free FQ on immobilized antibodies (**Figure 1**). Performances of this new immunosensor will be presented and the interest of the dual-working electrode design will be highlighted. Finally, the use of this immunosensor in real samples and the analysis of numerous commercialized samples from Perpignan will be exhibited.

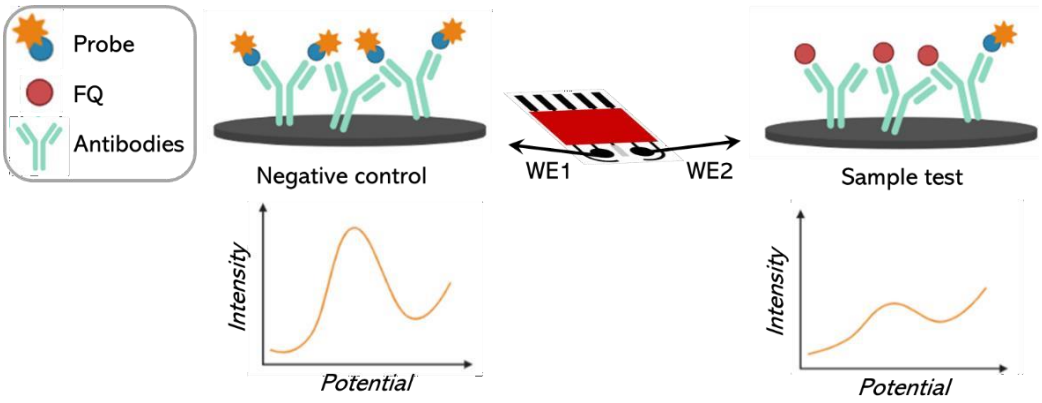

Figure 1. Detection principle of electrochemical dual immunosensor for EF detection

Strategies to increase the efficiency of photosynthetic microorganism-based Biophotovoltaic cells

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Biophotovoltaic cells (BPVs) offer an interesting alternative for the transformation of light to electricity if an efficiency comparable to photovoltaics could be achieved. However, the power efficiencies of current BPV devices[1] are still far below than the maximum estimated achievable[2]. Thus, this work focuses on strategies to overcome the main current limitations of these devices, i.e., cell-to electrode electron transfer and microorganism exoelectrogenesis. Green microalgae *C. vulgaris* were immobilized on electrodes modified with poly (3-aminophenylboronic acid), whose boronic acid residues bind covalently to cis-diols, like saccharides present in cell surfaces[3]. Microalgae were exposed to different stress factors (drought and nutrient starvation). Chronoamperometries and Pulse Amplitude Modulation (PAM) fluorometry were performed simultaneously to assess the photocurrent generation, the photosynthetic efficiency, and the stress. Cells immobilized under dry conditions on boronic acid-modified electrodes yield a 40.6-fold increase in photocurrent (1.976 ± 0.654 µA cm^{-2}) compared to cells immobilized on bare electrodes. This photocurrent density is the highest obtained from *C. vulgaris* to date. Furthermore, cells immobilized under wet conditions did not yield any photocurrent. These results suggest that: i) the cell-to-electrode electron transfer might be improved by binding phototrophs to electrodes using structures that mimic mechanisms for cell-to-surface or cell-to-cell adhesion and, ii) stressing photosynthetic microorganisms, specially by desiccation, induces alternative pathways to dissipate excess of energy that result in an increased exoelectrogenesis.

This work has been carried out with financial support from King Abdulaziz University collaborative project "Sustainable Biorefineries for the Future of Chemical Industry" (contract number: TT16008). ZHM acknowledges the support by a Martí Franquès scholarship of the Department of Chemical Engineering of the URV (Ref:2018PMF-PIPF-27).

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Electrochemical detection of viable Neuroblastoma (Neuro-2a) cells using a tetrazolium salt

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Marine neurotoxins are natural products, some of them extremely potent, responsible for serious seafood poisoning events. Examples of emerging marine toxins are ciguatoxins (CTXs), produced by microalgae of the genera *Gambierdiscus* and *Fukuyoa*, and tetrodotoxins (TTXs), produced by bacteria. Those neurotoxins have specific mechanisms of action that will ultimately determine their toxicological effect. CTXs and TTXs have been demonstrated to bind to and modulate the activity of cell membrane voltage-gated sodium channels (VGSCs). CTXs bock these channels in an open state, whilst TTXs block them in a closed state.

These toxins usually produce a change in the physiology, the morphology and/or the viability of cells, which can be detected and quantified by cell-based assays (CBAs). Most CBAs require the presence of agonists or antagonists, e.g. the drugs veratridine and ouabain, to counteract or emphasize the action of those toxins. The colorimetric assay most commonly used to determine the number of viable cells requires 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT). When cells are viable, this tetrazolium salt is reduced to the insoluble and redox active formazan dye by cellular dehydrogenases. Afterwards, dimethyl sulfoxide (DMSO) is added, which lyses the cells and solubilise the formazan crystals producing a coloured product, whose absorbance can be measured at 570 nm.

Our work is focused on the development of an electrochemical strategy for the detection of MTT, if possible not solubilized, for the final purpose of developing a cell-based biosensor for CTXs and TTXs. First, cells were immobilised on electrodes of different materials, i.e. poly(3,4-ethylenedioxythiophene (PEDOT), polyaniline, polylysine, gold and carbon. Then, cyclic voltammetry was used to measure the insoluble MTT precipitated on the electrodes. This strategy was used to investigate the viability and biocompatibility of immobilised cells on those electrodes. Other experimental parameters, such as cell density and incubation time, were also evaluated. Voltammetry detection of insoluble MTT seems a promising transduction strategy in future electrochemical cell-based biosensors.

Carborane- or Metallacarborane-Linked Nucleotides for Redox Labeling. Orthogonal Multipotential Coding of all Four DNA Bases for Electrochemical Analysis and Sequencing

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Electrochemical analysis of nucleic acids is an attractive and cost-effective technique, which is routinely used for the detection of particular DNA or RNA sequences. For most electrochemical applications, redox labeling of DNA bases is achieved through an attachment of some oxidizable or reducible molecules or functional groups with characteristic redox potentials, which need to be within a potential window compatible with the type of electrode used. We report the synthesis of a series of 2′-deoxyribonucleoside triphosphates bearing dicarba-nido-undecaborate ($[C_2B_9H_{11}]^{1-}$) or [3,3′-ironbis(1,2-dicarbollide)]$^-$ (FESAN, $[Fe(C_2B_9H_{11})_2]^{2-}$). The redox-modified DNA probes were prepared by primer extension (PEX) using tailed primers and were hybridized to capture oligonucleotides immobilized on gold or glassy carbon electrodes. The combination of nido-carborane- and FESAN-linked nucleotides with 7-ferrocenylethynyl-7-deaza-dATP and 7-deaza-dGTP allowed polymerase synthesis of DNA with all four fully modified, and each of the redox labels gave four differentiable and ratiometric voltametric signals. Thus, the combination of these four redox labels constitutes the first fully orthogonal redox coding of all four canonical nucleobases, which can be used for determination of nucleobase composition of short DNA stretches in one simple PEX experiment with electrochemical readout. [1]

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Spectroelectrochemical quantification of sunscreens

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Sunscreens are used in cosmetic products in order to protect the skin form UV light. Such radiations can damage and burn the skin, cause premature skin-aging and skin cancers[1]. The use of UV-filters is necessary for sun protection but can be damageable to marine organisms such as coral, as some UV-filters can contribute to coral bleaching[2]. Hawaii was the first American state to ban several UV-filters and was followed by other archipelagoes in the Pacific[3]. Today, the distribution of sunscreens containing either oxybenzone, octinoxate, octocrylene or avobenzone is prohibited in Hawaii[4]. In this study, we propose a novel analytical technique based on spectroelectrochemistry to identify and quantify three of those UV-filters present in most sunscreens sold in Europe. In spectroelectrochemitry, electrochemical and spectrophotometric measurements are done simultaneously. The proposed spectroelectrochemical method is a combination of UV spectroscopy, that studies the absorption of electromagnetic radiation in the UV region of the spectrum and chronoamperometry, which measures current intensity as a function of time by applying a constant difference of potential to the working electrode. Separately, spectroscopy is quite sensitive but does not enable for precise identification in case of mixture while electrochemistry enables identification of molecules in mixtures but lacks sensitivity. Spectroelectrochemistry takes advantage of both techniques for a more sensitive detection and an accurate identification.

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LATERAL AND VERTICAL FLOW ASSAYS

- **Jennifer Marfà** (UAB), “Biomimetic Polymers as Test Line in Nucleic Acid Lateral Flow Assays”

- **Liming Hu** (ICN), “Selection and Characterization of Bioreceptors to Develop Nanoparticle-Based Lateral-Flow Immunoassays under COVID-19 Pandemic”

- **Mireia Bernuz** (UAB), “Interferon Gamma Detection in Nucleic Acid Lateral Flow of Activated Lymphocytes”

- **Arnau Pallarès-Rusiñol** (UAB), “Vertical Flow Assay for Exosomes Characterization Based on their Intrinsic Enzyme Activity”

Biomimetic Polymers as Test Line in Nucleic Acid Lateral Flow Assays

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Molecularly Imprinted Polymers (MIPs) are synthetic materials mimicking biological receptors[1]. They are highly cross-linked macromolecular structures towards the template, which is then extracted after polymerization, originating cavities complementary to the template molecule[2]. Due to the wide range of applications, a molecularly imprinted polymer mimicking streptavidin, in which biotin is used as a template (biotin-MIP) and their downstream applications, are presented. The rational *in-silico* design, the synthesis and the characterization of the MIP towards biotin[3], as well as the integration as a test line in a lateral flow strip is described in this work, for the detection of double-tagged PCR amplicon[4]. The application of this NALF (nucleic acid lateral flow) assay is demonstrated for the waterborne pathogen *E. coli* as well as for the quantification of circulating tumor cells of breast cancer (**Figure 1**), respectively. This MIP is a cheaper and robust alternative to the affinity protein as the test line, presenting outstanding analytical features for their integration in lateral flow assays and the detection of a wide variety of targets.

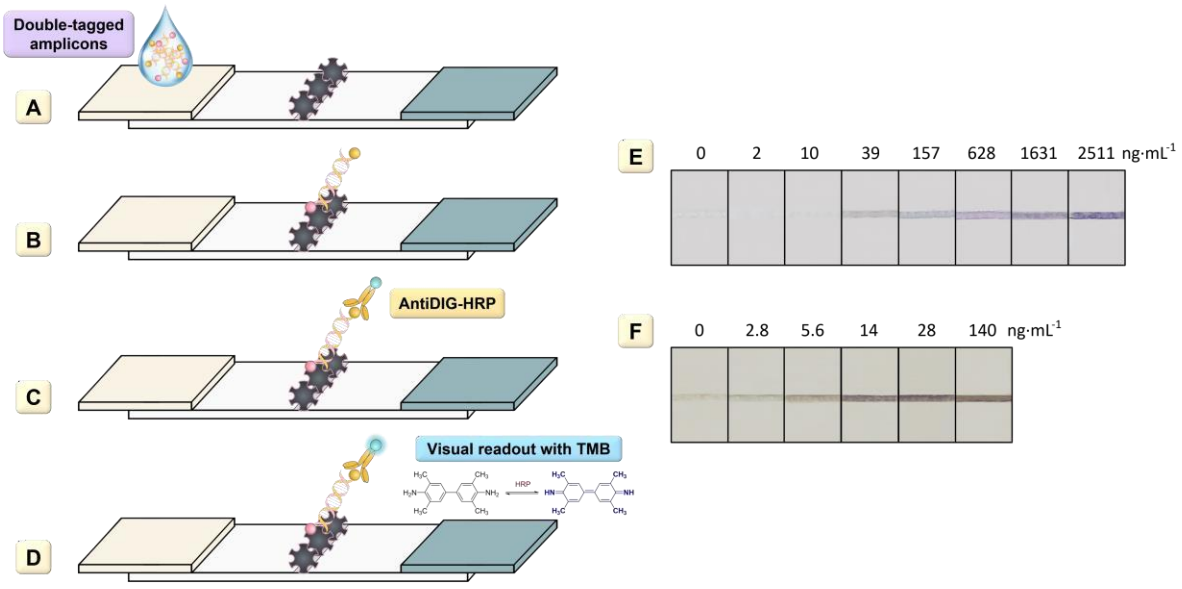

Figure 1. NALF design for detection of a double-tagged amplicon; A) Addition of the analyte (double-tagged amplicon) in the sample pad, B) Reaction of the MIP with BIO-tag of the amplicon, C) Addition of the specific antibody antiDIG-HRP to the sample pad to achieve the optical readout with the DIG-tag of the amplicon, and D) Visual readout using TMB. Results obtained for different concentrations of double-tagged amplicon ranging from E) 0 to 211 ng·mL^{-1} for *E. coli* and F) 0 to 140 ng·mL^{-1} for circulating tumor cells of breast cancer.

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Selection and characterization of bioreceptors to develop nanoparticle-based lateral-flow immunoassays under COVID-19 pandemic

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The COVID-19 outbreak has imposed the urgent need for rapid and low-cost diagnostic tools able to support high-frequency testing[1,2]. Among the different diagnostic platforms, lateral flow assay (LFA) allows the rapid and specific measurement of biomarkers in their clinical relevant range. Due to their advantages (fast, simple, low cost, and user-friendly etc.) WHO suggested their use to fight the COVID-19 outbreak and recognized people infected with SARS-CoV-2[3]. The selection of bioreceptors with strong affinity and high specificity is crucial in the development of lateral flow assays, especially in the sandwich ELISA format. Despite this, few companies provide these performance parameters of receptors, therefore, for developers the selection of suitable bioreceptors faces several experimental hurdles. Motivated by this, we developed LFA to detect SARS-CoV-2 nucleoprotein in saliva, and to achieve this we screened tens of antibody couples using two techniques: (1) enzyme-linked immunosorbent assay (ELISA), to quickly check antibody binding performance; and (2) the half-stick dot format (a simplified version of a LFA), to check their compatibility with the conditions encountered in a LFA. At the end of the process, only two couples of antibodies from 80 pairs tested on ELISA were actually suitable to work in a LFA format. The whole antibody selection required over 10 months and ~25,000 €, making it poorly effective during an emergency situation as the current COVID-19 pandemic. Therefore, we urge antibody producers and distributors to consider the implementation of more extensive characterization of their products, which would allow researchers to make better-informed purchases. We realize that a longer bioreceptor characterization implies higher costs for companies, nonetheless we truly believe that researchers would rather buy more-expensive, but well-characterized antibodies than cheaper but poorly-characterized ones.

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Interferon gamma detection in nucleic acid lateral flow of activated lymphocytes

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Interferon-γ (IFN- γ) is an inflammatory cytokine released mainly by activated T cells in response to intracellular pathogens, cancer and immune disorders (1). Therefore, IFN- γ expression level is a good biomarker of intracellular infections. The aim of this work it to develop a new IFN- γ transcript lateral flow assay. The main advantages of this method are the preconcentration of the T cells by anti-CD3 modified magnetic particles and the retrotranscription on oligo (dT) magnetic particles (2). Moreover, the stimulation time of the lymphocytes is reduced compared to the IFN-γ detection gold standard assay. The amplification of the cDNA is made on a unique assay doing a PCR. Furthermore, the lateral flow immunoassay also has a control line of the assay made with a biotinylated molecule. Therefore, the lateral flow has two lines; anti-fluorescein (for IFN- γ) and the control line. Previously in our group, the read out the PCR amplicons was made with an amperometric analysis (3). In this work the read out is made with golden particles and carbon particles modified with streptavidin and the comparison between them is made.

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Vertical flow assay for exosomes characterization based on their intrinsic enzyme activity

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Exosomes are nano-sized vesicles which are currently under intensive study as potential diagnostic biomarkers for many health disorders. Particularly, they are emerging as a new biomarker in liquid biopsies for cancer, since it is known that they are profusely released by cancer cells containing the neoplastic fingerprint of the primary tumor and cancer-specific DNA mutations.

Our group are currently focused on the development of rapid diagnostic tests (RDTs) for the analysis of exosomes as biomarkers of clinical interest. Among those, ELISA-based optical immunosensors were reported for the analysis of protein biomarkers located on exosomes membranes[1]. Beside this, the study of intrinsic Alkaline phosphatase (ALP) enzyme activity in exosomes was proposed for the use as biomarker of interest in metastatic cancer [2].

This work focuses on the design of a novel Vertical Flow Assay (VFA) based on enzymatic labelling and in different formats for the detection of exosomes from cell culture supernatants of hFOB (Human Fetal Osteoblasts) cell line, and SKBr3 and MDA metastasic breast cancer cell lines, as a model application, stablishing an approach to potentially use it as a rapid diagnostics test (RDTs) for cancer or other diseases. To achieve that, different approaches were explored, in all instances based on an enzymatic readout using the intrinsic activity of alkaline phosphatase. On one hand, and in order to simplify the analytical procedure, the presence of alkaline phosphatase (ALP) in the membrane of exosomes was directly addressed in order to provide the signal for the visual readout. On the other hand, the immunological detection of the exosomes by biotinylated antibodies in connection with streptavidin-ALP conjugated were used in order to take advantage of the high affinity of avidin-biotin interaction. After optimizing the protocol and designing the platforms to perform de VFA, the detection was simplified, and the visual readout of the exosomes were achieved. As a conclusion a promising approach was designed to detect specific biomarkers in cancer-derived exosomes.

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MICROSYSTEMS AND DEVICES

- **Elena Alberto Serrano** Disposable analytical microdevice for home monitoring of urea in blood

- **Meritxell Rovira** (CNM), “Validation of a Flow System for the Simultaneous Quantitative Analysis of Several Target Analytes in Sweat Samples”

- **Alex Pascual-Esco** (UAB), “Automatic Warning Microanalyser for Heavy Metals Monitoring in Natural Waters”

- **Josune J. Ezenarro** (CNM), “Screw-Like Photonic Sensor for in-Line Biofilm Monitoring in Water Networks”

- **Beatriz Rebollo-Calderón** (UAB), “Point-of-Care Microanalyzer for Monitoring Metabolic Hereditary Diseases”

- **Mireia Burdó-Masferrer** (CNM), “16s rRNA Hybridisation Assay for the Detection of Escherichia coli with a Portable and Automated Lab-on-a-Chip Platform”

Disposable analytical microdevice for home monitoring of urea in blood

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Urea is a nitrogenous organic compound found in blood and other body fluids. It is the degradation product of biomolecules that contain nitrogen in their structure, such as proteins. The level of urea in blood is used as a biomarker to control the renal system function. Since kidneys filter urea from blood, a malfunction is reflected in an urea concentration increase in the body, leading to a disorder called uremia.[1]

Drawing a parallel with diabetes and glucometers, miniaturized, portable, low-cost, automatic, highly robust and disposable analyzers with low sample and reagent consumption are required to allow a rapid determination and monitorization of urea in capillary blood by specialized personnel or even the patients themselves.

In this work, a versatile sensing platform based on cyclic olefin copolymer (COC) technology, incorporating a potentiometric detection system consisting of two ammonium ion selective electrodes (ISE), a gas-diffusion membrane and an enzyme, is designed, manufactured and characterized. The analysis strategy chosen consists in indirectly determining urea in the form of ammonium, obtained as a product of its enzymatic degradation by urease. A gas-diffusion membrane allows separation of the product (in the form of ammonia) from the rest of the sample matrix, avoiding possible interferences.

Chemical and kinetics variables of the analytical microsystem have been optimized, such as buffer composition, pH, enzyme concentration and analysis time, among others. Optimal working conditions have been achieved with a volume of 3 µL of 30 U/mL urease solution in 0.1 M HEPES buffer at pH 7, and an analysis time of 10 minutes.

Results obtained demonstrate that the developed disposable urea microdevice can be used to determine urea in the expected concentration range in real blood samples. Likewise, the developed versatile sensor platform can be used in the future as a basis to determine other different analytes that potentially generate ammonia/ammonium by other specific enzymatic reactions.

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Validation of a flow system for the simultaneous quantitative analysis of several target analytes in sweat samples

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This work reports on the development of a fluidic system for the quantitative analysis of different biomarkers in sweat. Five potentiometric ion-selective field-effect transistors (ISFETs) for pH, sodium, potassium and chloride were used. The system comprises a PMMA flow cell where six different sensors were integrated together with a reference electrode. External tubing and a peristaltic pump were used for driving the solutions into the flow cell (Figure 1A).

The system was tested with artificial sweat solutions showing a previously reported sweat composition. Different flow rates were tested in order to find the best condition for simultaneously calibrating the sensors. Sweat matrix effects were studied showing a very small change in the sensor responses when compared with those ones recorded in standard buffered solutions.

In order to analytically validate the system, 20 samples of real sweat were analyzed and results compared with those recorded by HPLC and commercial ion-selective electrodes (ISEs). Mean error values of the response of the sodium, potassium and chloride sensors compared with the HPLC were around 20% whereas the pH ISFET sensor showed an error of just 2 % when compared with the commercial ISE sensor (Figure 1B).

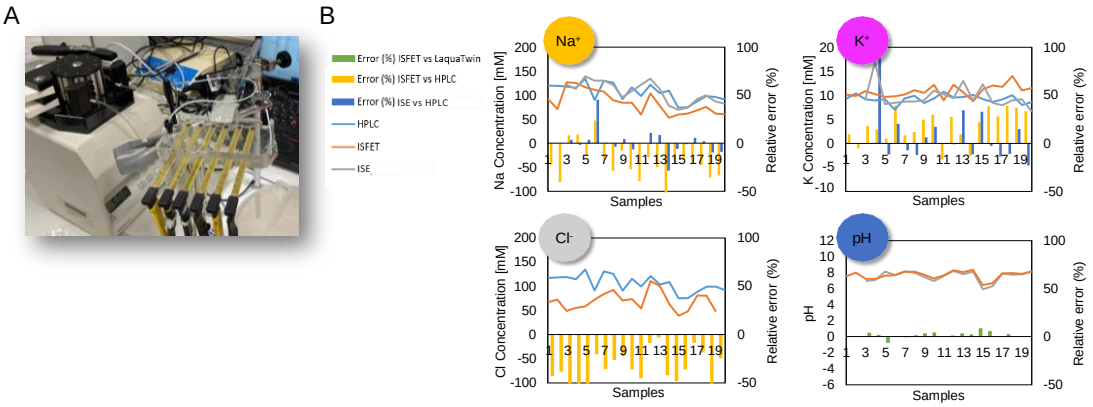

Figure 1. Concentrations obtained for 20 samples of sweat using different devices and relative errors comparing the different techniques.

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Automatic Warning Microanalyser for Heavy Metals Monitoring in Natural Waters

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Contamination of water by heavy metals has been a major environmental concern for decades. The techniques for heavy metals detection conventionally employed require bulky instrumentation and the analysis process is time-consuming [1]. The miniaturization of analytical procedures brings some important advantages for in-situ environmental continuous monitoring like enhanced portability and reduced reagents consumption and wastes. In order to enhance sensitivity and improve detection limits, the use of nanoparticles as analytical reagents is being assayed with optimal results. In this work, we present a strategy for the automatic monitoring of different heavy metals in water using Carbon Dots (CDs) as selective optical labels.

Microreactors based on Low-Temperature Co-fired Ceramics (LTCC) technology were developed to better control the synthetic conditions of CDs and thus improve synthesis reproducibility. They were fabricated by a multilayered approach technology and temperature is controlled by an embedded heater and a PT100 sensor [2]. A microfluidic platform was designed to perform the heavy metals automatic analysis by using Computer-Aided Design (CAD) software, and layers were micromachined onto Cyclic Olefin Copolymer (COC) substrate with a Computer Numerical Control micromilling machine. The platform was inserted in a customized miniaturized optical detection system.

For the detection of heavy metals, fluorescence quenching measurements were performed by applying a reverse Flow Injection Analysis approach, where the CDs are sequentially injected into MilliQ water (blank) and heavy metal standard solutions. Five different heavy metal ions (Co^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, and Pb^{2+}) were selectively detected. Limits of detection between 2 and 12 ppb were obtained for all the heavy metal ions. Spiked tap water samples and polluted soil samples were also analysed obtaining recovery rates of around 100% for the five heavy metals studied demonstrating the applicability of the proposal as a toxicity control system.

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Screw like photonic sensor for in-line biofilm monitoring in water networks

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Pipes for water distribution, refrigeration and industrial production are affected by the formation of microbial bio-layers, i.e. biofilm that can increase pipe corrosion and clogging, change organoleptic properties of water/products and act as focus of pathogenic microbial risk. In order to mitigate the risk to human health associated with water distribution. Herein we present the development of a photonic device which will allow to monitor the thickness and composition of the biofilms growing on the water pipes. The device (Figure 1 A) contains a reference channel to correct changes in the measurement associated with environmental factors, as well as several measurement channels with 50 µm guides in parallel that allow a very sensitive measurement of biofilm formation. Biofilms with different compositions and structures (*E.coli, p. putida, p. fluorescence, Shewanella, Serratia*, *S. aureus,* cyanobacteria, yeast) have been grown on the sensor surface and analyzed using spectroscopic techniques. Analyzing the chemical composition of the bacterial strains by their diffuse reflectance measurement in the UV-Vis-region and the morphology by backscattering, bacteria could be identified (Figure 1B). The screw like structure of the device and the good results make us envision this technology as a possible solution to the need of in-le sensors for continuous diagnostic of water networks.

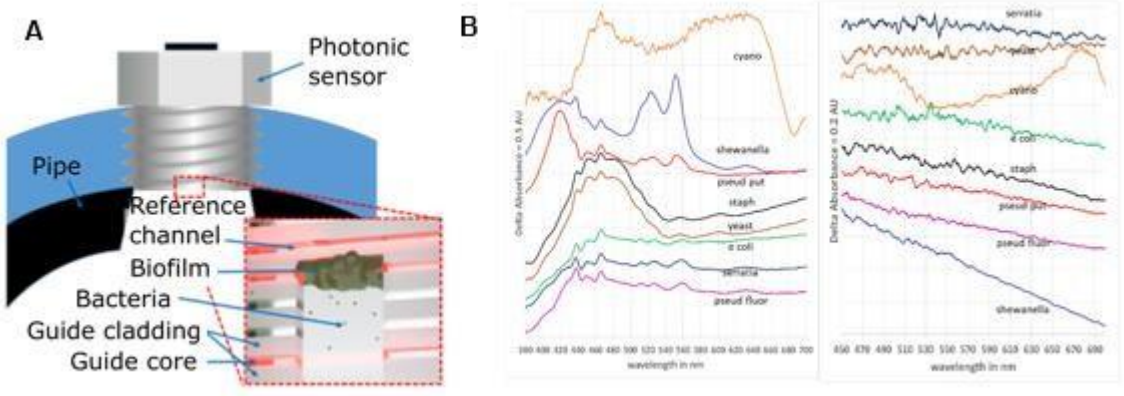

Figure 1. A) Illustration of architecture the screw-like optical sensor. B) Diffuse absorption spectra (left) and diffuse transmission spectra (right) of selected biofilms. For a better visualization, the spectra are shifted (see delta absorbance scale).

Point-of-care microanalyzer for monitoring metabolic hereditary diseases

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We have developed a Cyclic-Olefin-Copolymer (COC) microfluidic analyzer to be used as a point-of-care monitoring system for patients with metabolic hereditary diseases that present hyperammonemia [1]. This microanalytical device includes an ion selective electrode (ISE) and a screen printed reference electrode for the potentiometric determination of ammonium ion (NH_4^+), as well as a polyvinyldene fluoride (PVDF) gas diffusion membrane to isolate the analyte from the sample matrix [2] and a protective membrane to allow blood analysis and to lengthen the life of the device.

Two different conductive supports for the ISE and two different compositions for the ISE polymeric membrane have been evaluated. Results show that these parameters do not affect the analytical characteristics of the system, but the composition of the selective membrane does influence the life of the device, which reaches up to 28 days.

A wide range of protecting membranes made of different materials and porous sizes have been tested to select the appropriate, keeping suitable analytical characteristics for the detection of healthy and pathologic levels of NH_4^+ and, offering a proper protection from the blood proteins to avoid damage to the PVDF membrane. The developed microanalyzer showed a sensitivity of 62.82 $mV \cdot dec^{-1}$, a lineal range from 30 to 10000uM NH4+, a limit of detection of 18.58 µM NH_4^+ and an analysis time of less than 7 minutes for the highest concentration analyzed (1000µM NH_4^+), along with a good repeatability: RSD values of 3.28%. 3.33% and 0.65% for concentrations of 30, 100 and 1000µM NH_4^+, respectively.

Finally, plasma blood samples provided by the Sant Joan de Déu Hospital have been analyzed using both our potentiometric microanalyzer and an enzymatic spectrophotometric reference method. Results show good correlation when analyzing plasma samples (with an error lower than 10%) and a systematic underestimation when using our microanalyzer for analysis of blood samples, which can be corrected applying a correcting factor of 12%.

Therefore, we have demonstrated that our microanalyzer is an outstanding candidate to be used for bedside determination of NH_4^+ in blood that could be implemented in all types of clinics.

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16s rRNA hybridation assay for the detection of *Escherichia coli* with a portable and automated lab-on-a-chip platform

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Antimicrobial resistance is a growing worldwide problem. In the case of severe infections, antimicrobial agents are prescribed without an accurate diagnostic due to the lack of fast systems to identify the aetiological agent causing disease and its antimicrobial resistance profile. Therefore, there is a demand to develop new technologies able to fasten the diagnostic of infections in order to reduce the misuse of antimicrobial agents.

In this work, we developed a compact and automated lab-on-a-chip platform to quantify the amount of *Escherichia coli* in biological samples related to infection diseases. The platform includes a pumping, absorbance measurement and reagent reservoirs components together with a series of wax valves to control the flow (Figure 1a). A sandwich-like hybridization assay to detect the 16S rRNA sequence of *Escherichia coli* is implemented on our lab-on-a-chip. A microchannel-patterned glass support (Figure 1b) is used as the solid phase to carry out the assay. The limit of detection achieved with this platform for a range of concentrations of a commercial DNA sequence was 0,01nM (Figure 2).

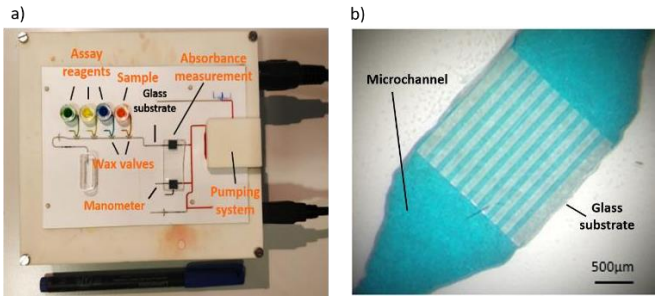

Figure 1. a) Lab-on-a-chip platform. b) Glass substrate in a microchannel.

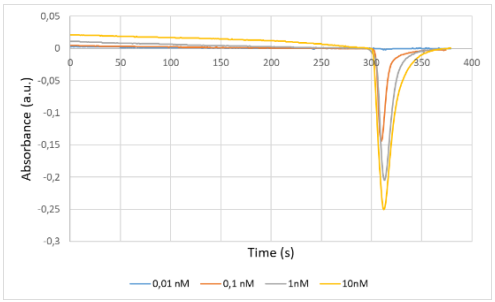

Figure 2. Absorbance values for 4 DNA concentrations.

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POSTERS

- **Catherine M. Lapada** (URV), “Development of Aptamer Assay for the Detection of Cylindrospermopsin in Environmental Water”

- **Christine Aubrey C. Justo** (URV), “Ultrasensitive Multiplex Detection of Microorganisms Related to Preterm Birth”

- **Laia L. Fernández** (UAB), “Impedimetric Sensor for Ibuprofen Based on Modified Composite Carbon-Paste Electrodes”

- **Núria Serrano** (UB), “Antimonene as a New 2D Layered Material for Voltammetric Sensors”

- **José Manuel Díaz-Cruz** (UB), “Sensitive Voltammetric Detection of Ni(II) Using a Low-Cost Commercial Screen-Printed Electrode”

- **Clara Pérez-Ràfols** (UB), “Discrimination of Beers by Using a Single Commercial Screen-Printed Electrode”

- **Qing Wang** (UAB), “Determination of Chemical Oxygen Demand (COD) Using Nanoparticle-Modified Voltammetric Sensors and Electronic Tongue Principles”

- **Greta Gaiani** (IRTA), “Ciguatoxin Detection in Fish, Algal and Environmental Samples Using a Sandwich Immunosensor”

- **Naga Adithya Chandra Pandurangi** (URV), “(Bio)Electrochemical Tools for Integration of Biological Systems and Electronic Components (BIONIC)”

- **Ankur Ruhela** (URV), “Development of a PCR-Based Bioassay for the Detection of HPV Using scCro DNA Binding Protein and Magnetic Beads”

- **Verena R. Schulze** (UAB), “Magnetic Solid Phase Extraction of Arsenic and Detection in Water Using Microfluidic Platforms”

Development of Aptamer Assay for the Detection of Cylindrospermopsin in Environmental Water

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Contamination of surface waters with the cyanotoxin cylindrospermopsin (CYN) is an increasing global concern due to its serious threat to human health and adverse impacts to the aquatic ecosystem. When ingested through untreated or insufficiently treated drinking water, CYN has the potential to inhibit protein synthesis and can be toxic to liver and kidney tissue. The goal of this work is to develop a rapid and sensitive aptamer-based assay for the detection of CYN in environmental water which can be employed for onsite monitoring. Aptamers against CYN have been selected from a highly diverse DNA library through Capture-SELEX, a selection strategy suitable for small molecular weight targets, whereas candidate sequences were identified using Next Generation Sequencing. The binding properties of the selected aptamer candidates are currently being evaluated using magnetic bead and plate Enzyme Linked Aptamer Assays (ELAA), which are based on CYN immobilized on different matrices. In parallel, displacement assays are being developed for CYN detection. The aptamer candidates are immobilized on matrices through hybridization with partially complementary probes and their displacement to the solution to enable CYN binding is monitored. The best performing aptamer in terms of binding affinity, specificity and potential structural change upon target binding will be chosen for the development of a simple and sensitive assay enabling the rapid visual detection of CYN in environmental water.

Ultrasensitive multiplex detection of microorganisms related to Preterm birth

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Preterm birth (PTB) or birth before 37 completed weeks of pregnancy is the world's leading cause of childhood mortality, recording about 15 million cases per year with about 1 million deaths in infants and children younger than five years old.[1] It has three clinical subtypes namely spontaneous PTB (45%), indicated PTB (30%), and preterm premature rupture of membranes (PPROM, 25%).[2] Women experiencing preterm labor and PPROM have been reported to typically harbor *Mycoplasma hominis* (MH), *Ureaplasma parvum* (UP), and *U. urealyticum* (UU) in their amniotic fluid, a normally sterile environment.[3] In here, we report an ultrasensitive multiplex detection assay for these mycoplasmas utilizing tailed primers in the multiplex polymerase chain reaction coupled with enzyme-linked oligonucleotide assay (mPCR-ELONA). The tailed primers and probes were designed to be specific to each of their targets. As proof-of-concept, the mPCR-ELONA's detection limits for MH, UP, and UU using synthetic single-stranded DNA were 860 aM, 156 aM, and 972 aM, respectively. MH, UP, and UU present in long-term stored clinical swab samples were also detected using the developed mPCR-ELONA.

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Impedimetric sensor for Ibuprofen based on modified composite carbon-paste electrodes

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Ibuprofen (IBP) is a nonsteroidal anti-inflammatory drug (NSAIDs). This drug is used worldwide to treat the inflammation, fever, and pain. But it also can have side effects like increase the chance to have heart, renal and hepatic insufficiency. In Spain, this drug doesn't need medical prescription to be obtained, so in this society people is making a massive use of this drug. In addition, not all the drug in metabolized and the remain one is expulsed by urine so can be found in wastewater [1][2] .

In this work, we use Electrochemical Impedance Spectroscopy (EIS) to detect the interaction between Hg nanoparticles (Hg-NPs) and the IBP, which is a more economical and easy way, compared to HPLC [3][4]. Composite electrodes based on graphite modified as Hg-NPs was used to apply this technique [5]. EIS is a complex technique that gives information about the ability of a circuit to resist the flow of electrical current. Usually, the data obtained is represented in Nyquist plot, and adjusted to an equivalent circuit.

The results obtained shows that the equivalent circuit has two parts: the first one remains constant, which means that give information about the electrode, and the second one gives information about the interaction between the Hg-NPs and the IBP. Which this information we can obtain a calibration curve with high sensitivity (19±0.7 $[\Omega\cdot(mg\cdot L^{-1})^{-1}]$ and r^2=0.989) and the linear range goes from 5 to 50 $mg\cdot L^{-1}$. A based-carbon sensor modified with Hg-NPs could be a cheap, easy to obtain, reliable, and robust device to detect IBP.

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Antimonene as a New 2D Layered Material for Voltammetric Sensors

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Nowadays the development of voltametric sensors with an enhanced response is a topic of study and interest. In this regard, along the last decades, 2D layered materials have arisen great attention due to their outstanding physical properties and excellent features (e.g. large surface area, great mobility, morphology tunability, and the possibility to change their surface properties) that make them valuable to be applied in energy storage, optoelectronics, catalysis, and sensors. Among them, antimonene is an emerging 2D material with a layered rhombohedral structure and with the highest degree of exfoliation, forming sheets of few-layer thicknesses and the lowest oxidation-to-bulk ratio as compared to the other 2D layered materials based on pnictogen elements, which could enhance its performance. However, its applications in voltammetric sensors have rarely been explored [1].

In this work, antimonene-based screen-printed electrodes (2D Sb_{exf}–SPE) were developed by drop-casting an exfoliated antimony suspension on the working electrode surface of both a screen-printed carbon electrode (SPCE) and a screen-printed carbon nanofibers modified electrode (SPCNFE). 2D Sb_{exf}–SPCE and 2D Sb_{exf}–SPCNFE were analytically assessed for the simultaneous voltammetric determination of Cd(II) and Pb(II) as model metal ions. The developed sensors were compared to each other and to the bare SPE in order to determine if the combination of 2D Sb_{exf} with carbon nanofibers results in a substrate that synergistically merge the exceptional properties of both materials. Moreover, the applicability of 2D Sb_{exf}–SPE for the detection of Cd(II) and Pb(II) was assessed in a natural sample.

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Sensitive Voltammetric Detection of Ni(II) Using a Low-Cost Commercial Screen-Printed Electrode

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Ni(II)-ion is a typical industrial pollutant that can be found in wastewaters with concentrations ranging from less than 1 µg L^{-1} to the mg L^{-1} level. Traditionally, nickel has been determined by adsorptive stripping voltammetry (AdSV) with dimethylglyoxime (DMG) in a NH_3/NH_4^+ buffer by using mercury electrodes [1]. With the progressive decay of mercury, adaptations of this method to bismuth and antimony film electrodes have been successfully tested [2], as well as the use of screen-printed electrodes modified with DMG [3].

In this work, a simpler and low-cost option is proposed by using directly an unmodified commercial screen-printed carbon electrode (SPCE) in a medium containing DMG and NH_3/NH_4^+ buffer. In contrast with the previous methods, the Ni(II)-DMG complex adsorbs directly on the screen-printed carbon surface, with no need of mercury, bismuth or antimony coatings [4].

The method yielded well-defined stripping peaks using a deposition time of 120 s, with a linear dependence of the peak area on the concentration of Ni(II) inside the range 1.7 - 150 µg L^{-1}. A very low limit of detection of 0.5 µg L^{-1} was computed under the same conditions, comparable or even better than those achieved with modified electrodes reported in the literature. An excellent reproducibility and repeatability with 0.3% (n=3) and 1.5% (n=20) relative standard deviation, respectively, were obtained. Moreover, the suitability of the method for the analysis of real samples was successfully assessed in a wastewater certificated reference material with notorious reproducibility and trueness.

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Discrimination of Beers by Using a Single Commercial Screen-Printed Electrode

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A simple, fast and costless methodology without sample pre-treatment is proposed for the discrimination of different types of beers. It is based on measurements by cyclic voltammetry (CV) using a commercial screen-printed carbon electrode (SPCE) without any modification. In order to account for the differences in the measurements made with successive screen-printed units, a correction is made with a standard sample of beer which is measured with every new SPCE unit. Data are explored with principal component analysis (PCA) and submitted to partial least squares discriminant analysis (PLS-DA) and support vector machine discriminant analysis (SVM-DA),

In a first approach, discrimination models were made with a limited set of beer brands which allowed a reasonable prediction and validation, quite better in the case of the non-linear method SVM-DA. Then, the methodology was applied to a bigger set of beer samples including standard brands (which were used for calibration and validation) and white-label beers from local supermarkets (which were submitted to the discrimination models to compare the type stated in the label with the model prediction). Consistent discrimination models were built and validated for 7 classes of beer (Lager, Toasted, Black, Alcohol-free, White beer, IPA and Stout) belonging to more than 30 brands. However, their application to *ca.* 40 white-label beers showed important differences between them for the same labelled type that made difficult their assignment to the predefined classes. This suggests that maybe the characteristics of white-label beers are somewhat more diffuse among types than in the case of the standard brands.

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Determination of Chemical Oxygen Demand (COD) Using Nanoparticle-Modified Voltammetric Sensors and Electronic Tongue Principles

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Chemical Oxygen Demand (COD) is a widely used parameter in analyzing and controlling the degree of pollution in water. COD is defined as the amount of molecular oxygen (in milligrams of O_2) required to decompose all the organic compounds in 1 L of aqueous solution to carbon dioxide and water. Electro-oxidizing the organic contaminants to completely transform them into CO_2 and H_2O using sensors is considered the best method for COD estimation. In this sense, copper electrodes have been reported based on the fact that copper in alkaline media acts as a powerful electrocatalyst for oxidation of aminoacids and carbohydrates, which are believed to be the major culprits for organic pollutions.

In this work, four electrodes were studied for COD analysis employing the cyclic voltammetry technique: Nafion film covered electrodeposited CuO/Cu nanoparticle electrode (**E1**), Cu nanoparticle-graphite-epoxy composite electrode (**E2**), CuO nanoparticle-graphite-epoxy composite electrode (**E3**) and Ni Cu alloy nanoparticle-graphite-epoxy composite electrode (**E4**). Glucose, glycine, potassium hydrogen phthalate (KHP) and ethylene glycol, which show different reducibilities, were chosen to be the standard substances to play the role of organic contaminants with different degradation difficulties. It was observed from the obtained cyclic voltammograms that glucose is very easy to be oxidized by those four electrodes and electrode **E1** shows the best performance, with a linear range of 19.2~1120.8 mg/L and limit of detection of 27.5 mg/L. Besides, KHP is very difficult to be oxidized by these four electrodes. Water samples were also analyzed with the electronic tongue array composed of these four electrodes based on the Principle Component Analysis (PCA) technique. As a result, the main component of river samples, which is easy or difficult to be degraded, can be evaluated by the PCA technique. This evaluation is very helpful for the accuracy of COD detection. The resulting sensor-based method demonstrates great potential not only for estimating the precise value of COD, but for predicting the difficulty behavior in its degradation, in a simple, fast, and clean methodology, which is completely suited to the present demands of green techniques.

Ciguatoxin detection in fish, algal and environmental samples using a sandwich immunosensor

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Ciguatera fish poisoning (CFP) is caused by the ingestion of fish containing ciguatoxins (CTXs), lipophilic marine toxins produced by microalgae of the genera *Gambierdiscus* and *Fukuyoa* that accumulate into fish flesh and through the food webs. CFP is one of the most relevant seafood-borne diseases worldwide and it is characterized by severe neurological, gastrointestinal, and cardiovascular disorders and affects approximately between 50,000 and 500,000 consumers annually worldwide. Although, the real incidence of CFP is difficult to ascertain, due to under-reporting and misdiagnosis.

In this work, three different monoclonal antibodies (mAbs), two capture (3G8, 10C9) and a detector (8H4), were merged in a sandwich configuration for the combined detection of two main groups of CTX congeners (CTX1B and CTX3C). At first, the applicability of the immunosensor has been demonstrated with the analysis of fish samples coming from La Réunion island, in which CTX-like activity was previously detected with mouse bioassay and cell-based assay, providing results that correlated with these tests. Then, a fish from Cyprus was analysed, and resulted positive for CTX. This finding represents the first report of a potentially ciguateric fish in the Mediterranean. Additionally, extracts from *Gambierdiscus* and *Fukuyoa* were screened, allowing the separate detection of the two groups of CTX congeners, and giving new information regarding the toxin production of the genera. Finally, microalgae epiphytic on macroalgae were collected in Majorca (Balearic Islands, Spain), extracted, and screened with the immunosensor. Results showed a CTX content similar to the one obtained in the Great Caribbean Region, area in which the Ciguatera is endemic. The developed bioanalytical tool is user-friendly, and can help to mitigate ciguatera risk, contributing to the protection of consumers health.

(Bio)Electrochemical Tools for Integration of Biological Systems and Electronic Components (BIONIC)

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Abstract:

Developing effective and conductive biocompatible interfaces is crucial for the integration of bioelectronic systems. This work focuses on fabricating a reliable bioelectronic interface with pyrroloquinoline quinone-dependent glucose dehydrogenase [PQQ-GDH, EC 1.1.5.2]. For creating such interface, either conductive polymers such as polyaniline or nanomaterials like CNT were proven as compatible materials for establishing electrical communication [1]. Hence, we propose to create a composite matrix consisting of both conductive polymers and nanomaterials which will prove as an effective interface. This configuration will be realized on the gold electrodes by low cost and miniaturize-able process called electropolymerisation, useful for manufacturing scale-up. Initially, 3-aminophenyl boronic acid (3-APBA) monomer has been chosen for electropolymerisation and electrochemical characterization studies have been conducted. The apparent diffusion coefficient of the polymerized gold electrode has been analysed in the presence of different mediators with cyclic voltammetry, chronoamperometry and electrochemical impedance measurements. Results indicate that the diffusion through the polymer is accelerated for positively charged species and delayed for negatively charged species. Following this, the polymer will be conjugated with carbon or gold nanoparticles and analysed for conductivity and permeability. The stability of this configuration at neutral pH and communication with PQQ-GDH will be optimized. Later, an attempt will be made to co-electropolymerize monomer, nanoparticle and PQQ-GDH selectively on micrometrically patterned electrodes. The resultant matrix can be used in biosensing, biofuel cells and bioelectronic interfaces as will be discussed.

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Development of a PCR-based bioassay for the detection of HPV using scCro DNA binding protein and magnetic beads

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Immobilization of biorecognition elements plays an important role in the development of sensitive biosensors. Specifically for PCR amplicon (DNA) detection, streptavidin-biotin bioaffinity interaction is used for immobilization to develop capture and reporter probes to perform bioassays. Along with streptavidin, DNA binding proteins (DBPs) can also be useful as an alternative of the streptavidin-biotin system. DBPs (such as scCro) can have high binding affinity to specific double stranded DNA sequences and incorporation of these specific DNA sequences into the primer sequences make them highly compatible with PCR based assays. More importantly, scCro does not bind to single stranded DNA (such as free primers that remain after a PCR reaction) as opposed to streptavidin that can bind to biotinylated primers, which can result in a reduction in the signal generated. By taking advantage of this DNA binding protein feature, a magnetic bead-based assay was developed to detect target PCR amplicon of Human papillomavirus. Signal generation was achieved with an HRP reporter conjugate. The proposed assay utilizing DBP-based capture probe shows higher sensitivity and is less affected by the presence of free primers compared to an equivalent streptavidin-based assay.