

NanoBiosensors SCHOOL

OCTOBER 17, 2023 - TIRANA, ALBANIA

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MAIN ORGANISER



Institut Català
de Nanociència
i Nanotecnologia



NanoBiosensors School 2023 Foreword

Nanobiosensors School aims to provide an overview on different topics based related to nanobiosensors, those sensors which include a biological sensing element and a transducer based or improved by nanotechnology, so any attendant will be able to learn the principles of different sensing techniques, sensors and devices, as well as the cutting-edge technological trends on this topic. The Nanobiosensors School is divided in four blocks:

- Zooming into biosensors: the nanoscale

Why to use nanomaterials on biosensors? In this block the properties and capabilities of nanotransducers and nanoreceptors are discussed.

- Paper-based biosensors: today and tomorrow

Paper stands as substrate for many biosensing platforms. What are the current paper-based biosensors in the market? How do they work? What can be expect in a near future?

- Advanced nanotechnology: biosystems and reading platforms

Biorobotics, fluorescence and electrochemical readers are the advanced systems to be discussed within this block.

- The potential of DNA: molecular biosensing

DNA bioreceptors stand over immunoassays due their reduced production costs, stability and versatility. Is molecular sensing the future of biosensors?

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NanoBiosensors School 2023 Speakers list

	page
Ruslán Álvarez (ICN2, Spain) Laser-Scribed rGO Electrodes Decorated with Metal Nanoparticles: Fabrication and Sensing Applications	4
Andy Jesus Bruno (ICN2, Spain) Laser-Scribed rGO Electrodes Decorated with Metal Nanoparticles: Fabrication and Sensing Applications	5
Maddalena Calabretta (University of Bologna, Italy) Bioluminescence-based bioanalytical tools	6
Francesco De Angelis (IIT, Italy) Plasmonic Solid State Nanopores for single biomolecule identification	7
Maria Guix (University of Barcelona, Spain) Sensing technologies integrated in biorobotic platforms: what and why	8
Petr Jakubec (Palacký University Olomouc /CATRIN, Czech Republic) Aptamers in Electrochemistry: Expanding Horizons in Biosensing, Bioassays, and Electrocatalysis	9
Leyla Karadurmus (Ankara University, Turkey) Recent advances of Molecularly Imprinted Polymers: Strategies for Electrochemical Methods on Pharmaceutical Analysis	10
Gabriel Maroli (ICN2, Spain) Building Your Device and App: A Guide for Pocketable Biosensors	11
Sibel A. Özkan (Ankara University, Turkey) Recent advances of Molecularly Imprinted Polymers: Strategies for Electrochemical Methods on Pharmaceutical Analysis	12
Andrew Piper (ICN2, Spain) Why Size Matters, Electrochemistry on the Nanoscale	13
Daniel Quesada (ICN2, Spain) Lateral flow assays design and components: a tutorial	14
Marianna Rossetti (ICN2, Spain) DNA-Based Biosensors for Protein Detection: Harnessing Structure- Switching and Scaffold Designs	15
Sümeysra Savas (University of Onyedi Eylül, Turkey) Detecting Pathogens in Real Samples Using Graphene Quantum Dots as Nanoenzymes: Is it Possible?	16

Laser-Scribed rGO Electrodes Decorated with Metal Nanoparticles: Fabrication and Sensing Applications

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Laser scribing techniques provide accurate and scalable methods for fabricating electrodes based on reduced graphene oxide (rGO). These techniques enable the creation of large surface area platforms that can also incorporate metal nanoparticles. The synergy between rGO and metal nanoparticles yields electrodes with enhanced electrical properties and surface area, making them ideal candidates for sensing and biosensing applications. This synergy arises not only from the laser's ability to provide the necessary energy for the photothermal reduction process or the unique chemical and physical properties of nanoparticle-decorated rGO, which is a nanostructured material, but also from the reduction process involving exfoliation, which imparts the necessary shape for subsequent transfer to a suitable substrate.

In this session, we will delve into the theoretical foundations supporting the use of graphene and its derivatives in sensing and biosensing applications. Additionally, we will explore how, with minimal materials and equipment, we can fabricate electrodes using a nanocomposite of rGO and gold nanoparticles. We will also discuss the exciting possibilities in terms of sensing capabilities and overall performance that can be achieved with these electrodes.

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- [3] DELLA PELLE, Flavio, et al. Freestanding laser-induced two dimensional heterostructures for self-contained paper-based sensors. *Nanoscale*, 2023, vol. 15, no 15, p. 7164-7175.

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Bioluminescence-based bioanalytical tools

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Bioluminescence (BL), that is the emission of cold light in living organisms, is a well-established optical detection technique explored and widely applied in several bioanalytical applications. BL provided a formidable manifold system relying on different luciferases and luciferin analogues with a wide range of applications spanning from molecular imaging to biosensing. It is highly attractive thanks to its intrinsic high-signal-to-noise ratio, high dynamic range, equipment simplicity, suitability to multiplexing. The availability of new BL proteins with tuned properties, both in terms of emission wavelength, kinetics and protein stability, is highly valuable in the bioanalytical field, with the potential to improve the sensitivity and analytical performance of the currently used methods for ATP detection, whole-cell biosensors, and viability assays among others [1,2].

Thanks to reporter gene technology a BL reporter protein can be expressed under the regulation of a target promoter sequence or enhancer elements, thus enabling correlation of reporter protein expression, measured as light signal, and transcriptional regulation. The BL reporter protein can be splitted into two halves for studying protein-protein interactions, exploiting the cDNA encoding for these fragments genetically fused to the two proteins which interaction is under investigation.

The ability to emit photons without the need of photoexcitation renders BL a suitable alternative to the more widespread fluorescence, and highly appealing for the implementation into portable and miniaturized devices. The unprecedented technological evolution of portable light detectors opened new possibilities to implement bioluminescence detection into miniaturized devices [3-6]. A portfolio of cell-based BL biosensors and cell-free systems will be presented with a look at the current challenges and the different strategies used to convert current laboratory methods into portable biosensors.

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Sequence identification of peptides and proteins is central to proteomics. Protein sequencing is mainly conducted by insensitive mass spectroscopy because proteins cannot be amplified, which hampers applications such as single-cell proteomics and precision medicine. The commercial success of portable nanopore sequencers for single DNA molecules has inspired extensive research on proteins based on electrical or optical readout. In this regard, a large variety of nanopores, both biological and solid state have been developed. The typical working principle consists in delivering DNA molecules into the pores and detecting the variations of ionic currents caused by the translocation of the molecule (in analogy with Coulter counter). Similarly, methods based on optical readouts have been developed. However, when moving from DNA to proteins some major challenges remain: (1) DNA bases are just 4 against the amino acids which are 20 hence their discrimination only by using electrical current levels or colorimetric readout is extremely difficult; (2) spatial and temporal resolution (sensitivity) to detect single amino acids within the same molecule; and (3) controlling the motion of proteins into the nanopores. In this context, the emergence of label-free optical analysis based on plasmonic enhancement shows great promises to address the first two challenges [1,2]. In fact, plasmonic nanopores can both confine and amplify the local electromagnetic field into the pore. The confinement improves the spatial resolution while the amplification helps to increase sensitivity. Notably, Raman spectroscopy provides unique molecular fingerprints to discriminate all the 20 amino acids [2]. We will present our latest results on plasmonic nanopores combined with Raman Spectroscopy for single-amino-acid identification [3,4]. In fig 1 is reported a sketch representing the concept: a gold plasmonic nanopore is fabricated on silicon nitride membrane (passing through). Molecules in solutions are delivered into the pore by means of electrophoresis and detected by plasmonic enhanced Raman scattering. In addition, we discuss the manipulation of molecule translocation and liquid flow in plasmonic nanopores for controlling molecule movement and for enabling high-resolution reading of protein/molecule sequences [3]. We envision that a combination of Raman spectroscopy with plasmonic nanopores can succeed in single-molecule protein sequencing in a label-free way.

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Figures

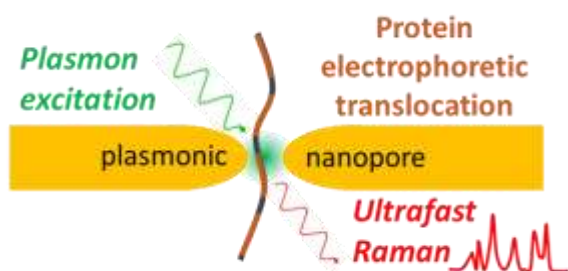


Figure 1: Sketch representing the concept of single molecule identification by means of Raman fingerprint.

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Soft robotic systems often present bio-mimicking designs that resemble actuation mechanisms of certain biological organisms, as for example in swimmers resembling fish or flagellated organisms. However, some unique properties from living organisms that are specially challenging to obtain in their artificial counterparts, such as self-healing, adaptability, or bio-sensing capabilities.[1] Several bio-hybrid robotics platforms across different scales had been developed,[2] but the ones based on living muscles has attracted increasing attention.[3] 3D printing technologies allowed the fabrication of advanced living robots based on skeletal muscle cells,[4] exploring new designs that are not bio-mimetic but really efficient, but also integrating nanomaterials for enhanced force output.[5] The integration of sensors in such platforms is key to envision a better understanding of the undergoing biological events, as well as going towards local actuation for improved guidance and manipulation. The key feature when designing these new generation of robots using living components as an active material coupled to sensing elements will be discusses, as well as the main challenges and applications, both in the biomedical and the environmental field

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Aptamers in Electrochemistry: Expanding Horizons in Biosensing, Bioassays, and Electrocatalysis

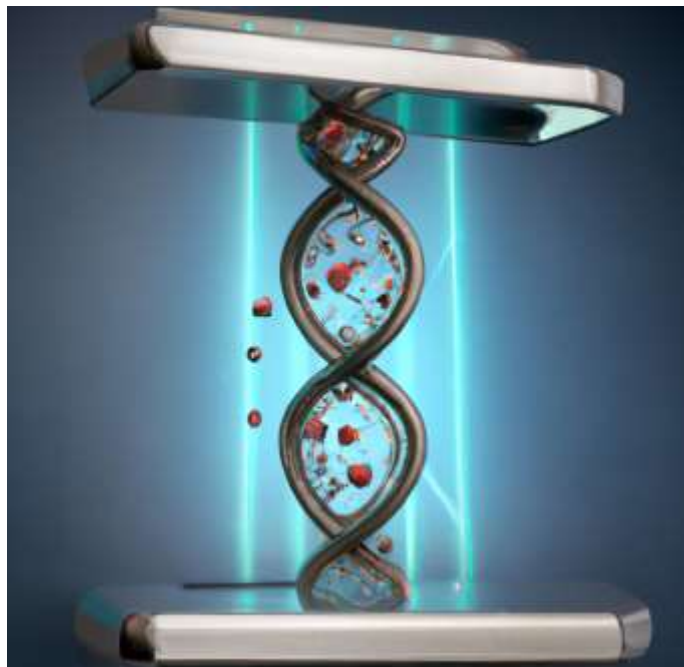
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Abstract

This lecture highlights the remarkable potential of aptamers, short single-stranded nucleic acids or peptides, as molecular recognition elements in the field of electrochemistry. Aptamers have gained significant attention due to their high affinity, specificity, ease of synthesis, and stability. Their application as recognition elements in electrochemical sensors allows for selective binding to target analytes, enabling the detection and quantification of various molecules, including small molecules, proteins, toxins, and whole cells. This has led to the development of ultrasensitive biosensors for applications in clinical diagnostics, environmental monitoring, and food safety. In addition, aptamers have emerged as powerful tools in electrochemical bioassays, facilitating the study of biomolecular interactions and dynamics. By combining aptamers with advanced techniques such as impedance spectroscopy, cyclic voltammetry, and chronoamperometry, researchers can investigate binding kinetics, thermodynamics, and conformational changes, thereby gaining valuable insights into aptamer behavior and complex biological processes. The advantages of aptamers, such as reproducibility, low batch-to-batch variation, and ease of modification, make them highly promising in electrochemistry. However, careful consideration of optimal immobilization strategies and sample matrices is essential to ensure reliable results. Overall, this lecture emphasizes the significant impact of aptamers in electrochemistry, paving the way for sensitive detection, comprehensive biomolecular analysis, and the development of efficient electrochemical systems.



Recent advances of Molecularly Imprinted Polymers: Strategies for Electrochemical Methods on Pharmaceutical Analysis

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Molecular imprinting technology, which forms molecularly imprinted polymers (MIPs), is a creative method that enables synthetic biorecognition gaps to imitate real biological derivatives like antibodies, receptors, enzymes, etc. [1]. After removing the target analyte, synthetic cavities enable the recognition and selective rebinding of the template. Although stable and durable MIPs seem relatively easy to create to achieve maximum efficiency, some optimization parameters should be considered, such as appropriate functional monomer and crosslinker and optimal ratios between functional monomer, template, and crosslinker [2]. In addition, the structure of the polymeric matrices and the type of bond contact between the template and the polymer are two important factors in MIPs [3]. The unique feature of superior selectivity of MIPs enables them to be used in various fields. Among them, MIP-based electrochemical sensors have a significant place because, with MIPs, it is possible to overcome the lack of selectivity issue in electrochemical sensors.

MIP-based electrochemical sensors and miniature electrochemical transducers can detect target analytes in situ. Thanks to superior chemical and physical stability, low-cost manufacturing, high selectivity, and fast response, MIPs have become an interesting field recently [4]. The studies on electrochemical MIP-based sensors to identify pharmaceuticals, heavy metals, hormones, enzymes, and biomarkers have grown. Moreover, without requiring time-consuming preparation procedures, these sensors have been successfully used in biological fluids (serum and urine samples) and pharmaceutical samples.

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Biosensors, comprised of the bioreceptor, transducer, and signal output components, have traditionally leaned towards using laboratory potentiostats for electrochemical applications, with limited exploration of portable alternatives. However, in today's landscape, individuals with basic electronics and programming knowledge can craft portable potentiostats, develop custom smartphone apps, and engineer compact devices. This school delves into the fabrication of pocketable devices. As a practical case study, we will showcase the development of an electrophoretic power supply tailored for paper-based sensors with wireless electrochemical readout—a project executed by our research group. We will highlight key points to consider during the design, how to control it in a wireless way through Bluetooth. Additionally, the design of antennas specifically tailored for NFC (Near Field Communication) potentiostats, considering factors like series resistance and geometry will be presented. Finally, in order to have a user-friendly pocketable device, we will provide an introductory overview of creating mobile applications using a block-based approach, without the need for advanced programming skills.

Recent advances of Molecularly Imprinted Polymers: Strategies for Electrochemical Methods on Pharmaceutical Analysis

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Why Size Matters, Electrochemistry on the Nanoscale

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Nanoscale electrodes have been a topic of intense research for many decades. Their enhanced sensitivities, born out of an improved signal-to-noise ratio as electrode dimensions decrease, make them ideal for the development of low-concentration analyte sensors. However, to date, nanoelectrode fabrication has typically required expensive equipment and exhaustive, time-consuming fabrication methods that have rendered them unsuitable for widespread use and commercialization. This talk will cover the fundamental theory behind nanoscale electrodes, how they can be fabricated, their current applications and future perspectives

Lateral flow assays design and components: a tutorial

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Having a diagnostic tool that can be quickly deployed, at large scale, of low-cost production and that can provide in situ and fast response is of great importance, as demonstrated during COVID-19 Pandemic. Rapid diagnostic tests, a.k.a. lateral flow tests, had a key role during SARS-CoV-2 outbreak as these biosensors are portable, easy-to-use, battery/equipment-free, affordable and provide a response which can be read by naked-eye in less than 10 minutes.

The objective of this tutorial is to provide an overview of the components of a lateral flow strip, the working principle of the assay, capabilities of the test and potential for diagnostic applications. Lateral flow assays are paper-based biosensors including nanomaterials as transducers (to produce the colorimetric signal), simple technology to use, but complex to develop with a lot of hidden potential and room for improvement

DNA-Based Biosensors for Protein Detection: Harnessing Structure-Switching and Scaffold Designs

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The accurate detection and monitoring of protein analytes for disease diagnosis and physiological tracking necessitate highly sensitive, specific, user-friendly, and rapid methods. Over the past decade, numerous DNA-based sensing techniques and sensors have been developed to achieve quantitative readouts of protein biomarkers. Inspired by the efficiency, specificity, and versatility of naturally occurring chemosensors reliant on structure-switching biomolecules, extensive endeavors have been made to emulate these mechanisms in artificial biosensor fabrication for protein detection. A promising alternative approach involves scaffold DNA biosensors, wherein diverse recognition elements (e.g., peptides, proteins, small molecules, antibodies) are precisely conjugated to the DNA scaffold to interact specifically with target proteins, exhibiting high affinity and specificity. These biosensors offer various advantages and hold immense potential, particularly in the substantial enhancement of transduction signals. In this presentation, I provide an overview of exemplary structure-switching-based and scaffold DNA sensors, while also introducing to the rational design of innovative sensing mechanisms and strategies based on programmable functional DNA systems for protein detection

Detecting Pathogens in Real Samples Using Graphene Quantum Dots as Nanoenzymes: Is it Possible?

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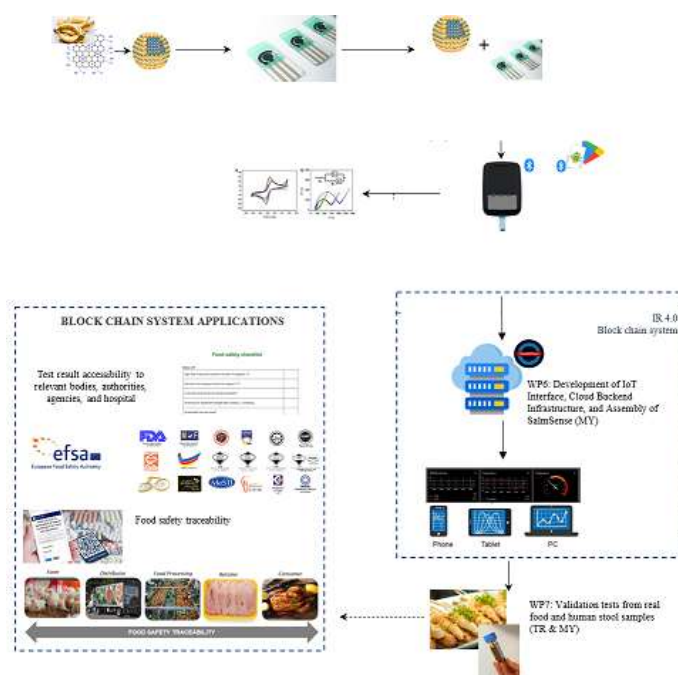
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Electrochemical sensors operate on the principle of detecting changes in electrical potentials resulting from chemical reactions [1]. These sensors enable precise, rapid, and specific measurements of target substances in samples, which is particularly advantageous for on-site detection of foodborne pathogens, contributing to the prevention of potential epidemics. The incorporation of nanomaterials in electrochemical sensors significantly enhances sensitivity and response time. Among these nanomaterials, Graphene Quantum Dots (GQDs) play a pivotal role. GQDs exhibit peroxidase (POD)-like catalytic properties, involving the oxidation of electron-donor substrates coupled with the simultaneous reduction of hydrogen peroxide (H_2O_2) [2,3]. Functioning as nanozymes, GQDs offer a label-free approach to analyte detection, replacing traditional HRP-based systems. Importantly, GQDs enhance the speed of electrochemical reactions, a critical consideration in the development of efficient electrochemical sensors. In this training, practical use of a GQDs based electrochemical sensor system will be demonstrated. This system is user-friendly, designed for smart mobile applications, allows label-free real-time direct pathogen measurement from actual samples.

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Figures



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