Società Chimica Italiana
Divisione di Chimica Analitica

UNIVERSITÀ DEGLI STUDI FIRENZE

GIORNATE DI BIOANALITICA

LA CHIMICA BIOANALITICA VERSO IL 2030

27 - 28 marzo 2023, Firenze

GIORNATE DEDICATE AI **PREMI**

"ALESSANDRO MANGIA"

E

"CRISTINA GIOVANNOLI"

E AL CONTRIBUTO DELLA CHIMICA BIOANALITICA

NEGLI OBIETTIVI DI EUROPA 2030

Giornate di Bioanalitica:
La Chimica Bioanalitica verso il 2030

FIRENZE

27-28 Marzo, 2023

Con il patrocinio dell'Università degli Studi di Firenze

ATTI

e

Programma finale

Società Chimica Italiana

Divisione di Chimica Analitica

Giornate di Bioanalitica 2023 – Gruppo Bioanalitica

ISBN: 978-88-94952-35-3

Editor: Società Chimica Italiana in coedizione con Divisione di Chimica Analitica e Gruppo Divisionale di Bioanalitica

27 Marzo 2023, Dipartimento di Chimica "Ugo Schiff", Sesto Fiorentino (FI)

Pubblicazione elettronica a cura di Sandra Furlanetto e Valentina Marassi

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Società Chimica Italiana
Divisione di Chimica Analitica

Giornate di Bioanalitica
Firenze, 27-28 marzo 2023
La Chimica Bioanalitica verso il 2030

Programma

• Lunedì, 27 Marzo 2023

10.30 – REGISTRAZIONE

11.30 – SALUTI ISTITUZIONALI - ALESSANDRA PETRUCCI, LUIGI MONDELLO

11.45 – **PREMIAZIONE MEDAGLIA "ALESSANDRO MANGIA" 2020, 2021, 2022, 2023**
Tavola rotonda con i vincitori del premio: "La bioanalitica verso il 2030" (A. Laganà, P. Reschiglian, M. Careri)
MODERANO: LAURA ANFOSSI, ALESSANDRO MANGIA, MARIA MINUNNI

12.30 – LIGHT LUNCH

13.15 – SESSIONE POSTER

SESSIONE I
MODERANO: CLAUDIO BAGGIANI, ALESSANDRO PORCHETTA

14.00 – **KN1** - Emerging sustainable approaches for nanomaterials-based (bio)sensing device manufacturing. F. DELLA PELLE, UNIVERSITÀ DI TERAMO - **PREMIO "GIOVANE RICERCATORE" 2020**

14.20 – **OC01** - The key role of ergothioneine in surface-enhanced Raman scattering spectra of biofluids. S. FORNASARO, UNIVERSITÀ DI TRIESTE
14.35 – **OC02** - HF5-multidetection as a fast, low volume and high-throughput method for isolation and characterization of biologically active extracellular vesicles from plasma of patients affected by Polycythemia Vera. S. GIORDANI, UNIVERSITÀ DI BOLOGNA
14.50 – **OC03** - Screening for toxic chemicals in food packaging and their detection in biological samples. F. PRATESI, FOOD CONTACT CENTER S.R.L

15.05 – **OC04** - Fast micro-extraction of N-Acyl-derivates in brain tissue by UHPLC-MS/MS. F. FANTI, UNIVERSITÀ DI TERAMO
15.20 – **OC05** - Bicyclic peptide-based Assay for Human Urokinase-type Plasminogen Activator (h-uPA) Cancer Biomarker: tools for Liquid Biopsy. G. MORO, UNIVERSITÀ DI VENEZIA

SESSIONE II

MODERA: DARIO COMPAGNONE

15.40 – PRESENTAZIONI FLASH

PF01 - Exosomes and their Cargo as "Two In One" Liquid Biopsy Tools for Cancer Biomarkers: New Generation of Luminescence-Based Biosensors Integrated with FFF Isolation From Biological Fluids. V. MARASSI, UNIVERSITY OF BOLOGNA
PF02 - IMMUNE-CRISPR: combining immunoassays with Cas12a-based detection using antibody-DNA conjugates. E. PAIALUNGA, UNIVERSITÀ DI ROMA "TOR VERGATA"
PF03 - NanoMIPs for thyroid hormones: an enantioselective challenge. V. TESTA, UNIVERSITÀ DI TORINO
PF04 - Set up and optimization of a LC/MS method for the characterization and control of mAbs. V. GHIZZANI, UNIVERSITÀ DI PAVIA
PF05 - Second-generation blood microsampling for LC-MS/MS analysis of THC, CBD and their metabolites. M. VENTURA, UNIVERSITÀ DI BOLOGNA
PF06 - A multi-tissue mercury determination in a commonly consumed elasmobranch (Mustelus mustelus) of the Mediterranean Sea. E. DÍAZ-DELGADO, STAZIONE ZOOLOGICA ANTON DOHRN, FANO
PF07 - Untargeted characterization of phenolic compounds for varietal and geographical classification of industrial hemp. E. TAGLIONI, UNIVERSITÀ DI ROMA "LA SAPIENZA"
PF08 - Chemical composition of European tea leaves: elemental content and health risk assessment for consumers. F. GIROLAMETTI, UNIVERSITÀ POLITECNICA DELLE MARCHE
PF09 - Electrochemical cell-free biosensors for antibody detection. S. BRACAGLIA, UNIVERSITÀ DI ROMA "TOR VERGATA"
PF10 - Analysis of Trace Elements: Eurasian Otter (Lutra lutra) an Expanding Sentinel Species in Friuli Venezia Giulia Region. M. VIT, UNIVERSITÀ DI UDINE
PF11 - Paper card-like electrochemical platform for point-of-care detection of glucose in tears. N. SEDDAOUI, UNIVERSITÀ DI ROMA "TOR VERGATA"
PF12 - Size separation and characterization of human polysomes by flow field-flow fractionation. A. ZATTONI, UNIVERSITÀ DI BOLOGNA
PF13 - A paper origami platform as a sustainable analytical approach for multiplexed analysis in urine. N. COLOZZA, UNIVERSITÀ DI ROMA "TOR VERGATA"
PF14 - Design of a printed electrochemical strip towards miRNA-21 detection in urine samples: optimization of the experimental procedures for real sample application. W. CIMMINO, UNIVERSITÀ DI NAPOLI "FEDERICO II"

PF15 - An Origami Paper-Based Biosensor for Allergen Detection by Chemiluminescence Immunoassay on Magnetic Microbeads. A. PACE, UNIVERSITÀ DI BOLOGNA
PF16 - A 3D-printed electrochemiluminescent enzymatic glucose biosensor based on luminol-H2O2 system and smartphone detection. D. CALABRIA, UNIVERSITÀ DI BOLOGNA

16.40 – COFFEE BREAK E SESSIONE POSTER

SESSIONE III
MODERANO: ANNA LAURA CAPRIOTTI, BARBARA RODA

17.10 – **KN2** - New perspectives of peptides: from nutraceuticals to diagnostics. C. M. MONTONE, UNIVERSITÀ DI ROMA "LA SAPIENZA" - **Premio "Giovane Ricercatore" 2021**

17.30 – **OC06** - An expanded framework for Swab Touch Spray-Mass Spectrometry towards food allergen analysis. L. TOMA, UNIVERSITÀ DI PARMA
17.45 – **OC07** - Microfluidics paper-based wearable electrochemical (bio)sensors for reliable detection of biomarkers in sweat. V. MAZZARACCHIO, UNIVERSITÀ DI ROMA "TOR VERGATA"
18.00 – **OC08** - Raman spectroscopic analysis of quality changes of lipoproteins in obese and healthy subjects. A. BONIZZI, ISTITUTI CLINICI SCIENTIFICI MAUGERI IRCCS DI PAVIA
18.15 – **OC09** - Optimization of a molecular beacon-based signal-off SERS biosensor for miRNA detection. S. TOMBELLI, CNR SESTO FIORENTINO (FI)
18.30 – **OC10** - Turning CRISPR-Cas12a Activity ON Using PAM-Engineered DNA Translator Responsive to Antibody. N. BAGHERI, UNIVERSITÀ DI ROMA "TOR VERGATA"

18.45 – APERICENA

• Martedì, 28 Marzo 2023

SESSIONE IV
MODERANO: SANDRA FURLANETTO, ROCCALDO SARDELLA

09.00 – **KN3** - Antibody-responsive DNA-based sensors for diagnostic applications. S. RANALLO, UNIVERSITÀ DI ROMA "TOR VERGATA" - **Premio "Cristina Giovannoli" 2022**

09.20 – **OC11** - Design of Immunoassays based on a Plant-Produced Antibody. T. SERRA, UNIVERSITÀ DI TORINO
09.35 – **OC12** - Robust analytical performances of a label-free electrochemical biosensor for the detection of Interleukin-6. R. CANCELLIERE, UNIVERSITÀ DI ROMA "TOR VERGATA"

9.50 – **OC13** - Novel hydrogel films integrating Prussian Blue nanoparticles and enzymes: a versatile strategy for portable electrochemical (bio)sensing. A. RAUCCI, UNIVERSITÀ DI NAPOLI
10.05 – **OC14** - In Vitro Inhibitory Effect of Green Tea Catechin Extracts against the SARS-CoV-2 Papain-like Protease Activity. E. AITA, UNIVERSITÀ DI ROMA LA SAPIENZA
10.20 – **OC15** - An origami paper-based chemiluminescent biosensor exploiting a structure-switching ATP-binding DNA aptamer. E. LAZZARINI, UNIVERSITÀ DI BOLOGNA
10.35 – **OC16** - Estradiol detection in water samples related to aquaculture exploiting plasmonic spoon-shaped biosensors. M. SEGGIO, UNIVERSITÀ DI VERONA
10.50 – **OC17** - Smartphone-based colorimetric paper sensor for food quality control based on biogenic amines monitoring. D. GREGUCCI, UNIVERSITÀ DI BOLOGNA

11.05 – COFFEE BREAK E SESSIONE POSTER

SESSIONE V
MODERANO: LAURA ANFOSSI, SANDRA FURLANETTO, ALESSANDRO PORCHETTA, BARBARA RODA

11.35 – **KN4** - Recent frontiers in lipidomics analyses by high-resolution mass spectrometry**.** A. CERRATO, UNIVERSITÀ DI ROMA LA SAPIENZA **- Premio "Cristina Giovannoli" 2023**

11.50 - **OC18** - From one-pot to tailor-made: an FFF-assisted analytical approach to develop high quality and ready-to-use conjugated gold nanoparticles for sensing applications. V. MARASSI, UNIVERSITÀ DI BOLOGNA
12.05 - **OC19** - Improving sensitivity and versatility of lateral flow immunoassays maintaining the full compliance to ASSURED criteria. S. CAVALERA, UNIVERSITÀ DI TORINO
12.20 – **OC20** - Metal nanoparticles laser patterning for cellulosic devices development. A. SCROCCARELLO, UNIVERSITÀ DI TERAMO
12.35 – **OC21** - Nanostructured electrochemical platforms: building biomimetic sensors for food applications**.** G. SELVOLINI, UNIVERSITÀ DI FIRENZE
12.50 – **OC22** - Solid-phase synthesis of nanoMIPs: how the parameters optimization can improve their binding ability**.** M. CHIARELLO, UNIVERSITÀ DI TORINO
13.05 – **OC23** - Thermogravimetry coupled with chemometrics for the differential diagnosis of hereditary hemolytic disorders. G. GULLIFA, UNIVERSITÀ DI ROMA "LA SAPIENZA"

13.20 – CHIUSURA E LIGHT LUNCH

COMUNICAZIONI POSTER

P01 BIOLUMINESCENCE ANALYTICAL METHOD BASED ON 3D MICROTISSUES FOR THE EVALUATION OF DIFFERENT BIOACTIVITIES OF WATER SAMPLES
MARIA MADDALENA CALABRETTA, Denise Gregucci, Marina Bonini, Elisa Neri, Martina Zangheri, Elisa Michelini

P02 A COMPREHENSIVE EVALUATION SCHEME AND QUALITY CONTROL AND PURIFICATION OF READY-TO-USE CONJUGATED GOLD NANOPARTICLES BASED ON ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION (AF4)
JUNJIE WANG, Stefano Giordani, Valentina Marassi, Barbara Roda, Pierluigi Reschiglian, Andrea Zattoni

P03 NATURAL NEUROTRANSMITTERS AS "GREEN" FUNCTIONAL MONOMERS FOR PROTEIN IMPRINTING
FEDERICA BATTAGLIA, Francesca Torrini, Pasquale Palladino, Simona Scarano, Maria Minunni

P04 MONOCLONAL ANTIBODIES (MABS) OPTICAL DETECTION BY COUPLING INNOVATIVE IMPRINTED BIOPOLYMERS AND MAGNETIC BEADS: THE CASE OF THERAPEUTIC MAB ANTI-MYOSTATIN DETECTION
Francesca Torrini, FEDERICA BATTAGLIA, Davide Sestaioni, Pasquale Palladino, Simona Scarano, Maria Minunni

P05 SYNTHESIS OF NANOPARTICLE-DECORATED BIODEGRADABLE MEMBRANES FOR USE IN ELECTRO-MEMBRANE EXTRACTION TECHNIQUES
BENEDETTA PASQUINI, Luca Marzullo, Serena Orlandini, Sandra Furlanetto, Cristina Román-Hidalgo, Adrián Vázquez-Romero, Mercedes Villar-Navarro, Germán López-Pérez, Mª Jesús Martín-Valero

P06 DESIGN FOR A PLATFORM FOR ANALYSIS AND SELECTIVE QUANTIFICATION OF THE AMOUNT OF UREA IN WASTEWATER AND FLUIDS OF BIOLOGICAL ORIGIN
LORENZO QUADRINI, Claudio Ciccone, Ilaria Palchetti

P07 DEVELOPMENT OF AN ANTIGEN LATERAL FLOW IMMUNOASSAY FOR THE ON-FIELD DETECTION OF AFRICAN SWINE FEVER VIRUS IN TARGET TISSUES
SIMONE CAVALERA, Barbara Colitti, Gianmario De Mia, Francesco Feliziani, Silvia Dei Giudici, Thea Serra, Valentina Testa, Matteo Chiarello, Fabio Di Nardo, Claudio Baggiani, Sergio Rosati, Laura Anfossi

P08 THREE STRATEGIES FOR THE MONITORING OF THE IMMUNE RESPONSE TO SARS-COV-2 BASED ON LATERAL FLOW IMMUNOASSAY
SIMONE CAVALERA, Barbara Colitti, Chiara Nogarol, Cristina Guiotto, Domenico Cosseddu, Thea Serra, Valentina Testa, Matteo Chiarello, Fabio Di Nardo, Claudio Baggiani, Sergio Rosati, Laura Anfossi

P09 SOLID-PHASE SYNTHESIS OF NANOMIPS: THE EFFECT OF DELAYED IMPRINTING ON THE POLYMERISATION
MATTEO CHIARELLO, Valentina Testa, Thea Serra, Fabio Di Nardo, Simone Cavalera, Laura Anfossi, Claudio Baggiani

P10 EVALUATION OF ALKALOIDS CONTENT IN LUPINUS ALBUS L. SAMPLES BY MEANS OF HPLC-MS/MS
FABIOLA EUGELIO, Sara Palmieri, Federico Fanti, Luana Messuri, Alessia Pepe, Dario Compagnone, Manuel Sergi

P11 FLUOROQUINOLONES IN HUMAN PLASMA AND THEIR UHPLC-PDA DETERMINATION USING ELECTROSPUN SORBENT FOR THE SOLID-PHASE EXTRACTION
Vincenzo Ferrone, Pantaleone Bruni, GIUSEPPE CARLUCCI

P12 LATERAL FLOW ASSAYS BASED ON DECORATED DNA SCAFFOLDS
SIMONE BRANNETTI, Serena Gentile, Alejandro Chamorro-Garcia, Erica Del Grosso, Francesco Ricci

P13 ONE-STEP ELIMC-BASED ASSAY FOR DETECTION OF TOTAL MICROCYSTINS AND NODULARINS IN WATER SAMPLES: SETTING-UP OF THE EXPERIMENTAL CONDITIONS FOR MODULATING SENSITIVITY
GIULIA VOLPE, Silvia Piermarini, Laura Fabiani, Danila Moscone

P14 EVALUATION OF THE ALTERATIONS IN MICE URINARY METABOLOMIC PROFILE FOLLOWING THE ADMINISTRATION OF OPIOIDS BY UHPLC-HRMS
Gaia Di Francesco, Martina Croce, Livia Gerecitano, FLAMINIA VINCENTI, Camilla Montesano, Matteo Marti, Manuel Sergi, Roberta Curini

P15 EXPLOITING PLATINUM NANOPARTICLES AS COLORIMETRIC LABEL IN LATERAL FLOW IMMUNOASSAY
FABIO DI NARDO, Simone Cavalera, Laura Anfossi, Claudio Baggiani

P16 SUSTAINABLE, COST-EFFECTIVE, AND FLEXIBLE SCREEN-PRINTED POTENTIOMETRIC SENSOR FOR RELIABLE ION CHLORIDE DETECTION IN SWEAT
ACHREF CHEBIL, Luca Fiore, Noemi Colozza, Vincenzo Mazzaracchio, Fabiana Arduini

P17 DESIGN AND OPTIMIZATION OF AN ON-FIBER CHEMILUMINESCENCE BIOSENSING SYSTEM
Cosimo Trono, SARA TOMBELLI, Simone Berneschi, Ambra Giannetti, Francesco Baldini, Donato Calabria, Massimo Guardigli, Mara Mirasoli

P18 SMART SENSOR FOR LITHIUM DRUG MONITORING DETECTION
ILARIA ANTONIA VITALE, Giulia Selvolini, Giovanna Marrazza

P19 AN IMPEDIMETRIC APPROACH TO DETECT B-LACTOGLOBULIN IN MILK-FREE PRODUCTS
GIULIA SELVOLINI, Niran Öykü Erdoğan, Ilaria Antonia Vitale, Gözde Aydoğdu Tiğ, Giovanna Marrazza

P20 INNOVATIVE COMBINATION OF THERMAL DESORPTION WITH ON-LINE SOLID PHASE EXTRACTION REVERSED PHASE LIQUID CHROMATOGRAPHY APPLIED TO TARGETED NUTRIMETABOLOMICS IN HUMAN BIOFLUIDS
Lapo Renai, Luca Marzullo, GIULIA BONACCORSO, Serena Orlandini, Massimo Del Bubba

P21 AF4 COUPLED TO CHEMOMETRICS FOR THE ANALYSIS OF PROTEIN FRACTION OF MILK
NICHOLAS KASSOUF, Pierluigi Reschiglian, Barbara Roda, Andrea Zattoni, Alessandro Zappi, Pietro Morozzi, Dora Melucci, Valentina Marassi

P22 A NEW MICROSAMPLING AND ENANTIOSELECTIVE LIQUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY APPROACH FOR THE BIOANALYSIS OF NOVEL PSYCHOACTIVE SUBSTANCES
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P23 A GUANOSINE-DERIVED SUPRAMOLECULAR HYDROGEL WITH DNAZYME-LIKE PEROXIDASE ACTIVITY AS A NOVEL PLATFORM FOR HYDROGEN PEROXIDE DETECTION
ILARIA TROZZI, Donato Calabria, Elisa Lazzarini, Andrea Pace, Silvia Pieraccini, Stefano Masiero, Mara Mirasoli

P24 THE BIOLUMINESCENT RECOMBINANT PROTEIN JAGGED1-FLUC AS POTENTIAL DIAGNOSTIC TOOL FOR THE HIGH- THROUGHPUT SCREENING OF COLORECTAL CANCER
ANGELA PUNZO, Alessia Silla, Patrizia Simoni, Antonio Pannuti, Barbara Roda, Aldo Roda, Sylvia Daunert, Cristiana Caliceti

P25 CHEMICAL CHARACTERIZATION OF A DEEP IONIAN SEA SEDIMENT CORE
MATTEO FANELLI, Serenella Zampatti, Federico Girolametti, Behixhe Ajdini, Anna Annibaldi, Cristina Truzzi, Silvia Illuminati, Federico Spagnoli

P26 EXPLORING THE DIAGNOSTIC POTENTIAL OF CHEMOMETRIC FINGERPRINTING FROM FFF-SORTED BIOLOGICAL SAMPLES TO TRACK AND DETECT EXOSOMAL POPULATION IN CANCER PATIENTS' SERUM
PLACCI ANNA, Valentina Marassi, Barbara Roda, Stefano Giordani, Andrea Zattoni, Pierluigi Reschiglian, Angela Punzo, Cristiana Caliceti, Aldo Roda, Benedetta Pasquini, Serena Orlandini, Sandra Furlanetto

P27 UNTARGETED METABOLOMICS STRATEGY BASED ON ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY–HIGH-RESOLUTION-ION MOBILITY-MASS SPECTROMETRY FOR CEREAL QUALITY AND TRACEABILITY
Nicolò Riboni, Maurizio Piergiovanni, FEDERICA BIANCHI, Monica Mattarozzi, Mariolina Gullì, Marina Caldara, Nelson Marmiroli, Maria Careri

P28 ELECTROCHEMICAL GENOASSAY ON MAGNETIC BEADS FOR ULTRA-SENSITIVE DETECTION OF KRAS ONCOGENE MUTATION ASSOCIATED WITH COLORECTAL CANCER IN LIQUID BIOPSY
Simone Fortunati, Chiara Giliberti, Roberto Corradini, Andrea Rozzi, Martina Neri, Alessandro Bertucci, MARCO GIANNETTO, Maria Careri

P29 PRELIMINARY INVESTIGATION ON THE COMBINATION OF GC-MS AND HPLC-UV/PDA METHODOLOGIES FOR MONITORING HONEYBEE (APIS MELLIFERA) METABOLIC AND FORAGING STATUS
RITA NASTI, Valeria Leone, Luca Giupponi, Annamaria Giorgi, Giangiacomo Beretta

Keynotes

Premio “Giovane Ricercatore”

Premio “Cristina Giovannoli”

KN1

Emerging sustainable approaches for nanomaterials-based (bio)sensing device manufacturing

Flavio Della Pelle

Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Campus "Aurelio Saliceti" via R. Balzarini 1, 64100, Teramo, Italy

Nowadays, the routine use of commercial screen-printed electrodes has become a reality in the electro(bio)analytical scenario; despite being highly widespread, they present some limitations regarding their default design, rigid substrate, and analytical performance. In a parallel track, the on-course green revolution pumps towards the use of natural compounds instead of synthesis compounds as well as to reduce the use of organic solvents. In this contest, emerging and green-produced nanomaterials (NMs) and nanocomposites, offer captivating opportunities to achieve competitive performances taking advantage of their catalytic and electroanalytical features, as well as their ability to improve the integration of biorecognition elements and mediators. In particular, the fabrication of tailored analytical devices integrating NMs and NMs-based conductive films is still a hot topic; to overcome tedious, expensive, and not sustainable conventional manufacturing techniques, several efforts are devoted to implementing effective and affordable technologies to produce nanostructured analytical devices. In this framework, low-cost substrates/materials and emerging manufacturing technologies, still represent a captivating and sustainable opportunity, and although much progress has been made, there are still unexplored rooms.

This presentation will be focused on our experience in using functional NMs, mainly produced by avoiding the use of solvents and pollutant chemicals, as building blocks for the implementation of completely lab-made analytical devices. An overview of nanomaterials' sustainable production, nano architectures assembling, and their integration into freestanding conductive films, flexible sensors and biosensors, and all-in-one devices will be given. The manufacturing and use of 0D, 1D, and 2D NMs including among others biochar, laser-induced graphene, group VI transition metal dichalcogenides (i.e., MoS_2, WS_2, $MoSe_2$, and WSe_2), and different nanocomposites will be presented. Particular attention will be paid to (i) nanomaterials preparation avoiding the use of solvents and (ii) fabrication of devices integrating NMs using low-cost substrates (i.e., thermoplastic, polymeric sheets, paper, nitrocellulose, etc.) and benchtop microfabrication technologies (i.e., stencil printing, laser scribing, cutter plotting, thermal lamination, etc.). Finally, the practical use of the developed NMs-based devices will be also covered concerning different sensing and biosensing applications.

The main goal of this presentation is to prove how NMs produced using emerging sustainable strategies can be easily integrated into tailorable cutting-edge analytical devices manufactured via accessible instrumentation.

This research was funded by the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy - VITALITY

KN2

New perspectives of peptides: from nutraceuticals to diagnostics

Carmela Maria Montone

Dipartimento di Chimica, Università degli studi di Roma La Sapienza, Piazzale Aldo Moro 5, 00185 Roma

Peptides are of extreme interest in food and clinical research fields. From a nutraceutical point of view, peptides are most versatile with several functionalities, including antioxidant, antihypertensive, antiproliferative/ anticancer, antimicrobial, and anti-inflammatory. Although the origins of peptide nutraceuticals are different (from various sources, e.g., plant, animal, microbial, etc.), their vital role in the contribution of nutrients and prevention of diseases is widely studied. Most bioactive peptide nutraceuticals are isolated from protein precursors by digestive enzymes during food processing, storage, or in vitro hydrolysis by several proteolytic enzymes. This lecture will present some recent applications of the role of short and medium-chain peptides. In particular, a dedicated analytical platform based on a purification step by size exclusion chromatography or ultrafiltration membrane and high-resolution mass spectrometry will be discussed to isolate and comprehensively characterize short-chain peptides. Short-chain peptides became particularly interesting and showed advantages over longer peptide sequences. In fact, short peptides have low cytotoxicity, and the ability to maintain their biological properties unaltered upon absorption is not subject to in-vivo transformation. Moreover, they can reveal multifunctional properties demonstrating excellent candidates for developing nutraceutical and functional foods helpful in preventing metabolic syndrome and other common diseases [1].

Together with standard peptides containing exclusively proteinogenic peptides, post-translationally modified peptides are of great interest, especially in biomarker discovery. Protein post-translational modifications (PTMs) currently represent one of the main challenges with proteomic analysis due to the important biological role they play within cells. Among more than 300 known PTMs, a limited number are extensively studied by proteomics technologies. Modified proteins often play important roles; however, such proteins are expressed in small amounts, usually not enough for analysis in global protein characterizations, and may be present in different forms simultaneously.

Moreover, some modifications decrease ionization efficiency and hinder MS identification; thus, studying a selected PTM requires specific purification and enrichment procedures. In this context, the selective enrichment of modified peptides produced during shotgun proteomics workflows is necessary for a comprehensive proteomic analysis. In this lecture, protein tyrosine O-sulfation, a PTM currently scarcely investigated for its low abundance and poor stability of the sulfate modification, will be presented. A detailed description of the progress achieved in sulfate detection and localization will be widely discussed [2].

[1] J. Peng, H. Zhang, H. Niu, & R. Wu, TrAC Trends Anal Chem, 2020. 125115835 10.1016/j.trac.2020.115835

[2] A.L. Capriotti, A. Cerrato, A. Laganà, C.M. Montone, S. Piovesana, R. Zenezini Chiozzi, C. Cavaliere. Anal. Chem. 92, 2020, 7964–7971, 10.1021/acs.analchem.0c01342

KN3

Antibody-responsive DNA-based sensors for diagnostic applications

Simona Ranallo

Dipartimento di Scienze e Tecnologie Chimiche, Università degli Studi di Roma Tor Vergata, Via della Ricerca Scientifica 1, 00133 Roma

Antibodies are considered among the most important clinically-relevant class of proteins and their detection and monitoring play a pivotal role in the diagnosis of a wide range of pathologies, including infectious and autoimmune disease. Current methods for the detection of specific antibodies are either rapid and easy to use but only qualitative or semi-quantitative (i.e., lateral flow immunoassays) or quantitative but laboratory-bound processes (i.e., ELISA, Western Blots, etc.) thus limiting their value at the point-of-care. Due to the above considerations, better analytical tools that allow the rapid, inexpensive, and quantitative measurement of antibodies are urgently needed. Recently, a new impulse in that direction has been given by nucleic acid nanotechnology that employs synthetic nucleic strands (DNA and RNA) as engineering material to create functional devices with nanoscale quasi-Angstrom precision. In this scenario, our research group has exploited the high programmability, low-cost, ease of synthesis, biocompatibility and chemical versatility of synthetic nucleic strands to design and develop programmable sensing nanodevices. More specifically, we have reported different examples of optical and electrochemical DNA-based sensors and circuits that allow the rapid and quantitative detection of different antibodies in an orthogonal fashion (REF: Nat Com 2017, Nat Com 2019, ACS Sensors).[1-3] More recently, we have also combined the advantages of synthetic nucleic acids (i.e., programmability of interactions and chemical versatility) together with the features offered by cell-free transcription systems (i.e., high sensitivity and low sample volumes) to develop a new class of biosensors that may be of utility in a wide range of applications, including point-of-care diagnostics (REF x2).

[1] S. Ranallo, C. Prévost-Tremblay, A. Idili, A. Vallée-Bélisle, F. Ricci. Nat. Commun. 2017, 8, 15150.
[2] S. Ranallo, D. Sorrentino, F. Ricci. Nat. Commun. 2019, 10, 5509.
[3] S. Bracaglia, S. Ranallo, K.W. Plaxco, F. Ricci. ACS Sensors 2021, 6, 6, 2442–2448.
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KN4

Recent frontiers in lipidomics analyses by high-resolution mass spectrometry

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Lipidomics, once considered a branch of metabolomics, has nowadays gained its proper analytical approaches that differ significantly from routinary methods for metabolomics. Shotgun mass spectrometry (MS) and liquid chromatography coupled to MS are the foremost techniques for lipidomics either in a targeted or untargeted fashion. In untargeted lipidomics, the structural information is highly dependent on the analytical methods with a progressively higher degree of confidence from the individuation of the lipid classes and subclasses to the evaluation of the stereochemical properties [1]. The recent trends in lipidomics generally follow two interconnected paths: (i) aiming at a more straightforward and automatized lipid identification and (ii) widening the structural information that can be obtained by MS-based lipidomics. As such, data processing and identification by software programs are toughened by a large number of adducts and several isomeric mass overlaps, that occur any time different lipid species generate adducts with the same sum composition [1]. Moreover, by the sole MS or LC-MS, it is difficult in practice to obtain structural information beyond the molecular lipid level, i.e., the knowledge of the lipid class, subclass, and fatty acid composition, with no further regiochemical and stereochemical evaluation. The issue of the isomeric mass overlaps has long been debated by the Lipidomics Standard Initiative, a community-wide effort that aims to create guidelines for the major lipidomic workflows including sample collection as well as data treatment, and represents a hurdle for correct lipid identification from MS data. Several approaches have been proposed for solving these mass overlaps, including a buffer modification workflow approach that takes advantage of the physicochemical and ionization properties of phosphocholine-containing lipids for dealing with their MS-based identification [2]. On the other hand, the evaluation of double bond positions in fatty acyl chains has always been of great concern since relevant studies have pointed out their significance in the chemical and biochemical role of lipids [3]. In the latest years, several innovative approaches for pinpointing carbon-carbon double bonds have been proposed, such as alternative dissociation techniques, ozone-induced dissociation inside the mass spectrometer, and chemical derivatization before analysis. Paternò-Büchi (PB) reactions of fatty acids with ketones have been successfully proposed for pinpointing double bonds in fatty acids, as PB-derivatized lipids generate diagnostic product ions under CID fragmentation in positive ion mode [1]. Our research group has recently proposed a complementary alternative to PB reaction for negative ion mode analysis based on an aza-Paternò-Büchi (aPB) reaction of lipids with 6-azauracil, that allowed enhancing the ionization based on its functional groups [4].

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Comunicazioni orali

OC01

The key role of ergothioneine in surface-enhanced Raman scattering spectra of biofluids

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Surface-enhanced Raman scattering (SERS) spectroscopy has lately attracted attention in the field of liquid biopsy as a quick and reasonably inexpensive technology that could dramatically improve clinical diagnosis and prognosis. SERS spectra, in fact, provide information about a collection of metabolites present in the studied biofluid, providing biochemical insight into specific health status. Ergothioneine, an unusual dietary amino acid containing the imidazole-2-thione substructure, is important because it is one of the few metabolites in biofluids that can be detected directly (without recognition elements) by SERS. In the past decade, many studies characterizing biofluids or other biological samples have unknowingly linked this amino acid with crucial metabolic processes, including inflammation, in a variety of disorders. With some exceptions, most studies on serum or plasma reported a higher relative amount of ergothioneine in control samples with respect to those of patients affected by a disease. However, because the SERS spectrum of ergothioneine was only recently reported by this group, most previous investigations mistakenly ascribed what are now recognized as spectral bands of this chemical to other compounds.

This presentation will summarize and re-evaluate the knowledge about the role of this compound in the so-called “label-free” SERS spectra of biofluids, in order to better understand the role of this compound in numerous clinical conditions.

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OC02

HF5-multidetection as a fast, low volume and high-throughput method for isolation and characterization of biologically active extracellular vesicles from plasma of patients affected by Polycythemia Vera

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Extracellular vehicles (EVs) are an heterogeneous group of bilayer membrane nanoparticles that play roles in the intracellular communication of both heathy and diseased cells as well as in the diagnosis and prognosis of various types of cancer [1]. Isolation and characterization of EVs from plasma is challenging due to their low concentration, high heterogeneity as well as the presence of nanoscale contaminants in their environment. The current main strategies exploited to isolate EVs (ultracentrifugation, size-exclusion chromatography and ultrafiltration) suffers from a series of problems such as requiring high amount of sample, providing little sample characterization, low time and purity efficiency, moreover they may affect the structural integrity and biological activity of the separated analytes [2]. Within this context we describe an innovative approach to isolate biologically active EVs from low amount plasma (40 µL/subject)) exploiting an Hollow Fiber Flow Field Flow Fractionation Multidetection (HF5-multidetection) platform. Our miniaturized device allowed for a fast (<25min) separation and a simultaneous spectroscopic size and shape characterization of the analytes while providing high-throughput, a soft and native separation and minimal dilution of the separated fractions collected at the end of the platform. In this work plasma samples from both healthy donors and patients affected by Polycythemia Vera (PV), a clonal disorder of hemopoietic stem cell characterized by chronic inflammation, were analysed and characterized by the online detectors (DAD, MALS, FLD) coupled to the separation system. EV-enriched fractions resulting from the HF5 separation were collected, concentrated by ultrafiltration (100 kDa ultrafilters) and further characterized by offline techniques to evaluate the size, biological activity, purity and marker expression of the isolated EVs . The isolated EVs from PV patients were spherical and expressed both EV-specific and immune, tumour, and senescence specific markers while Western blot highlighted the absence of common contaminants such as albumin. Overall, these results suggest that the analytical platform exploited can be an important asset in unravelling the role of EVs in orchestrating the complex interplay between PV tumour cells and the inflammatory and senescent microenvironment which is still is poorly understood.

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OC03

Screening for toxic chemicals in food packaging and their detection in biological samples

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Plasticizers as phthalates and bisphenols are widely used in the production of foodpackaging material (FCM) that can also contain non-intentionally added substances (NIAS) derived from degradation processes and/or impurities present in raw materials used for production. These chemicals, potentially toxic, can be transferred by contact from packaging into food and, consequently, taken up by humans. Thus, the purpose of this study was to investigate presence of plasticizers and their metabolites in packaging and in human urine samples through high sensitive advanced analytical techniques.
Presence of non-volatile and polar compounds (bisphenols and other NIAS) in packaging samples (i.e., papers, boards, plastics) was investigated by UHPLC-Q-TOF system (Sciex), using electrospray ionization in positive and negative mode. The analysis of volatile and semi-volatile compounds (phthalates and other NIAS) was made by GC-MS (Shimadzu). The analysis was conducted in 128 samples for BPA and BPS BPF and 59 samples for DEHP. Detection of BPA, BPS, BPF and DEHP metabolites in urine sample requires treatment with Abalonase for enzymatic deconjugation since they are excreted as glucuronide form. Sample were purified with C18 SPE. DEHP metabolites were analyzed by UHPLC-TOF-MS (Agilent), while bisphenols were analyzed with GC-MS/MS (Agilent) after derivatization with BSTFA 1% TMCS. Urinary concentrations were quantified using labelled internal standards added to the sample before enzymatic deconjugation and normalized for creatinine concentrations to take into account urinary excretion rate. The analysis was conducted in 30 urine samples obtained from subjects with type 2 diabetes, as part of a research protocol, who signed an informed consent form. Packaging: the results showed that recycled paper represents the largest source of DEHP, BPA and BPS that were present in more than 80% of the tested samples compared to none in reference material. BPF could not be detected. Presence of additional substances like 2- methyl-4-isothiazolin-3-one, alkylamine ethoxylated, 1,2 Benzisothiazol 2 (2H)-one were identified by untargeted analysis in plastic and paper samples.
Human urine samples: the results showed that there is a large exposure to these substances, since DEHP metabolites [1], BPA, and BPF were detected in all urine samples and BPS in the majority. Among bisphenols, BPA showed the highest concentrations followed by BPS and BPF and the same trend was found in the food packaging. Analyses of urine human samples showed that the exposure to plasticizers is relevant and among environmental sources recycled paper represents an important source of these chemicals that are known to act as endocrine disruptors and thus are potentially toxic for human health.

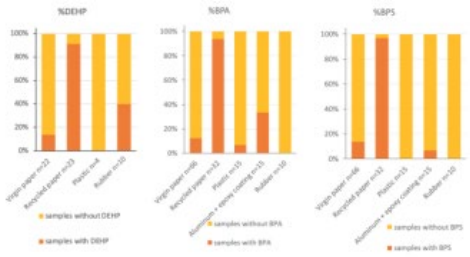

Figure 1: % of packaging samples resulted positive to presence of DEHP, BPA, BPS. (LOQ =0,05 mg/kg).

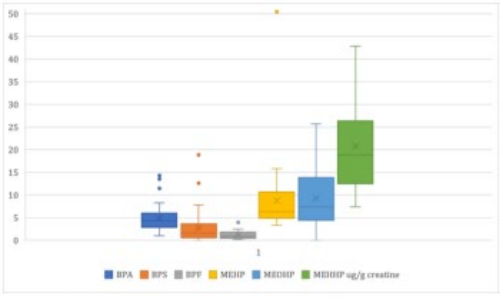

Figure 2: Concentration of BPA, BPS, BPF and DEHP metabolites normalized to creatinine in urine samples.

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OC04

Fast micro-extraction of N-Acyl-derivates in brain tissue by UHPLC-MS/MS

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Endocannabinoid system (ECS) was widely studied due to implication on several pathology condition and contribution on homeostasis balance, due to its biochemical functions acting on various system. ECS explicates its action mainly through specific neurotransmitters called endocannabinoids (eCBs) [1], but other compounds, structurally related to the major eCBs, were identified as "cannabimimetic" due to their capability to bind, fully or partially, CB1 and CB2, the main ECS receptors, or to inhibit eCBs degradation process. In this scenario new ECS related compounds called N-arachidonoyl conjugated eCBs raised as important neurotransmitters and modulators of classic eCBs levels, which derives from the conjugation between arachidonic acid and other bioactive molecules [2]. The determination eCBs in biological samples is not straightforward and the extraction usually requires large amount of sample by means liquid/solid extraction or solid phase extraction (SPE) and their low concentration in some biological matrices makes them a challenging analytical task [3]. The present study encompasses the development of a fast and reliable analytical method to quantify the main endocannabinoids and some of their conjugated congeners, particularly N-arachidonoyl amino acids, in brain tissue. Samples were homogenized with a fast and reliable procedure, then a micro solid phase extraction (µSPE) was developed for brain homogenate clean-up. Miniaturized SPE was selected as it allowed to work with reduced sample amount, while maintaining high sensitivity; this last feature was mandatory due to the low concentration of endocannabinoids in brain tissue that makes their determination a challenging analytical task. UHPLC-MS/MS was used for the analysis as it provided a great sensitivity, especially for conjugated forms that required the detection by negative ionization: polarity switching was therefore applied during the run, providing limits of quantification between 0.003 ng g^{-1} and 0.5 ng g^{-1}. This method provided also low matrix effect and good extraction recoveries from brain samples. To the best of our knowledge, this is the first time that µSPE is applied on this matrix for this class of compounds. The method was validated according to international guidelines, and then tested on real cerebellum samples from mice, sub-chronically treated with URB597, a well-known inhibitor of the fatty acid amide hydrolase [4].

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OC05

Bicyclic peptide-based Assay for Human Urokinase-type Plasminogen Activator (h-uPA) Cancer Biomarker: tools for Liquid Biopsy

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In the era of liquid biopsy, electrochemical biosensors can offer highly performing, user-friendly, cost-affordable, and potentially scalable solutions for the frequent monitoring of several types of cancer biomarkers, ranging from proteins up to exosomes. In this frame, peptides can find several applications from antifouling agents to bioreceptors enabling high selective and sensitive recognition of specific analytes at subnanomolar levels [1]. In particular, bicyclic peptides were successfully selected against the biomarker human Urokinase-Type Plasminogen Activator (h-uPA) [2]. The h-uPA is involved in the plasminogen activator system together with the urokinase-type plasminogen activator receptor and plasminogen activator inhibitors 1 and 2. This system is an extracellular enzyme complex with physiological functions and high levels of h-uPA can be associated with metastatic events and shorter survival times [3]. The affinity of these peptides was found to be 1000 times higher than those of other serine proteases. Recently, two bicyclic peptides (namely P_1 and P_2) were *ad-hoc* synthesized and tested as bioreceptors to develop a sandwich-type affinity electrochemical assay for h-uPA [4]. Both peptides were immobilized on streptavidin-coated magnetic microbeads (MBs), thus enabling the capture of the analyte even in complex biological matrices such as serum or plasma. Once mixed with the h-uPA spiked samples, the peptide-functionalized MBs were subsequently incubated with a primary (Ab_1) antibody, which selectively binds to h-uPA, and then with a secondary antibody (Ab_2) that recognizes the former and carries the enzyme alkaline phosphatase (AP), thus providing the biosensing platform. The presence of the conjugate at the MBs was followed immobilizing these latter at disposable screen-printed electrodes (SPE). The concentration of h-uPA was correlated to the oxidation of 1-naphtol, used as the substrate of AP, recorded by differential pulse voltammetry. The biosensing platform was further tested with portable instrumentation showing the potential and adaptability of this sensing architecture. Apart from enabling h-uPA quantification at nanomolar levels (60 ng/mL for P_1 and 10 ng/mL for P_2), this affinity-type assay showed the potential of bicyclic peptides applicability in sensing even in plasma. A third peptide, namely P_3, was introduced afterwards showing a higher sensitivity that P_2 in the electrochemical assay describe above. With the aim of testing and comparing different biosensing architecture, streptavidin-modified SPE were functionalized with biotinylated P_3. The changes upon incubation of different h-uPA concentrations were followed via electrochemical impedance spectroscopy in presence of a redox probe. To avoid time-consuming data elaboration, the variation of the Bode plots were considered, as previously reported [5]. The faradic assay provided promising results and the two biosensing strategies were further compared considering their capability to determine h-uPA in plasma and fulfill liquid biopsy requirement.

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OC06

An expanded framework for Swab Touch Spray-Mass Spectrometry towards food allergen analysis

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Swab Touch Spray - Mass Spectrometry (Swab TS-MS) is an ambient ionization mass spectrometry (AIMS) technique, which is characterized by the direct ionization of the sample without sample preparation or chromatographic separation, enabling high-throughput, simple and fast analysis. Swab TS allows *in situ* sampling of materials through a metallic nasopharyngeal swab from which ions are generated by an electrospray-like process once it is put in front of an atmospheric pressure interface of the mass spectrometer and after solvent and potential application (Figure 1) [1].

So far, this technique has found several applications in clinical and forensic fields, where the swab tips are used for sampling material from human tissues [2] or surfaces contaminated by organic gunshot residues [3]. In this context, the present study is aimed at expanding the application framework of Swab TS-MS towards protein analysis with a focus on food allergens. Food producers, restaurants and canteens have to implement proper cleaning procedures on production lines and food preparation surfaces to ensure proper protein removal. The continuous monitoring of the cleaning process implies swabbing sampling strategies coupled to screening analytical methods [4]. Screening assays, for example lateral flow assays, can improve traceability but often lack the required reliability to guarantee compliance. Here we present Swab TS-MS as an innovative strategy for rapid investigation of allergen residuals on food contact materials for secure compliance testing, using lysozyme from chicken egg white as a case study. A Q-Exactive mass analyzer was used for high resolution mass spectrometry acquisition in product ion scan, with a mass accuracy below 5 ppm. Peptides from tryptic digestion were targeted and experimental conditions such as geometrical parameters and solvent composition were studied. Then, the peptides giving the best response were selected for the assessment of analytical quality parameters. The signal was acquired over time, with peptides remaining detectable up to 20 minutes, thanks to the constant flow of solvent wetting the swab during the analysis. Good performance in terms of detection limits at low ppm level was observed under MS/MS conditions. An approach for *in situ* digestion to be performed directly on a contaminated surface was developed in order to obtain tryptic peptides directly on the swab tips after swab sampling. Finally, the developed swab TS-MS method was applied for the detection of surface contamination with egg white powder resulting in the detection of lysozyme target peptides. This method creates a fast and secure food safety compliance testing paradigm that can benefit the industry and consumer alike. offering a viable analytical alternative to common colorimetric or immunochemical assays used for cleaning validation and testing.

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OC07

Microfluidics paper-based wearable electrochemical (bio)sensors for reliable detection of biomarkers in sweat

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Recently, we highlighted in a review entitled "Electrochemical paper-based devices: when the simple replacement of the support to print eco-designed electrodes radically improves the features of the electrochemical devices" [1] the advantages of using paper-based layers "for vertical microfluidics or as a reservoir to deliver smart electrochemical (bio)sensors able to i) contain the reagents, ii) preconcentrate the target analyte, and iii) synthesize the nanomaterials inside the paper network" [1]. Herein, we report how we have exploited the foldability, capillary-driven flow, and reservoir features of the paper for the development of smart wearable devices for pH, Na^+, and cortisol detection. For instance, in the case of cortisol detection, an immunosensor washing-free wearable device was designed and fabricated by using different types of paper, an origami configuration, and magnetic beads to easily incorporate the primary antibody within the paper-based microfluidics. To render, the entire device wearable, the paper-based device was combined with a flexible NFC wireless module, delivering a cost-effective and battery-less wearable analytical tool. In this case, cortisol detection in standard solution was assessed with linearity in the range 10-140 ng/mL. To evaluate the accuracy, a recovery study was carried out in sweat samples at two levels, namely 20 and 40 ng/mL, obtaining a recovery equal to 100 ± 6 % and 98 ± 4 %, respectively. Finally, this device was successfully applied to determine the cortisol in sweat during the cycling activity of a volunteer, demonstrating its reliability. In the case of pH and Na^+ detection, we started from our previous works [2,3] based on the i) wireless and flexible epidermal device for pH monitoring in sweat and ii) carbon black-based sensor for the detection of Na^+, for the punctual detection of Na^+ and pH in continuous mode by using a flexible Bluetooth-based potentiometric paper-based multiple sensing wearable devices [4,5].

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OC08

Raman spectroscopic analysis of quality changes of lipoproteins in obese and healthy subjects

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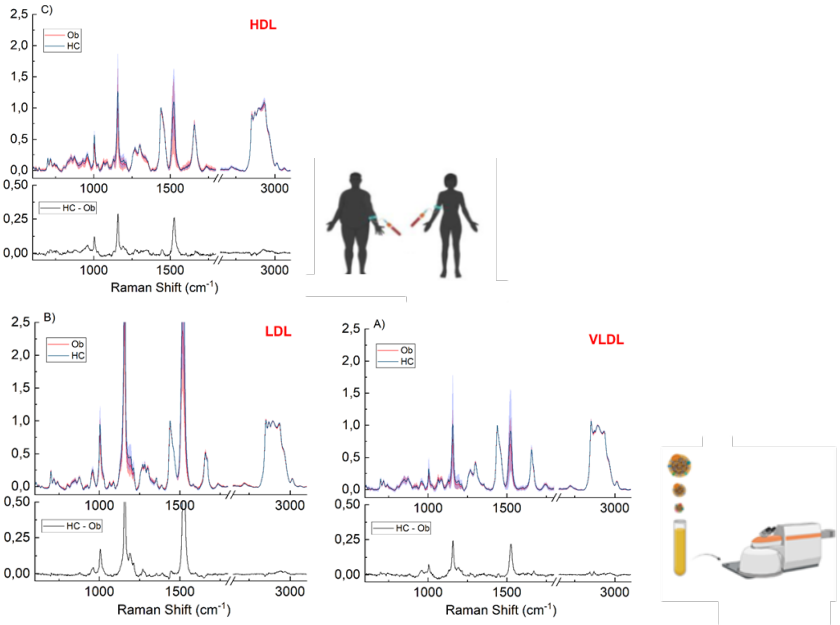
Lipoproteins (LPs) are macromolecular complex particles of lipids and proteins which are related to the transport of lipids through the body. Currently, the amount of cholesterol carried by LPs is one of the main parameters used in the clinical practice to assess the risk of cardiovascular events[1]. However, this measurement does not fully capture the composition and function complexity of LPs which are closely related to LP's *quality*, an emerging parameter that could improve the definition of cardiovascular risk[2]. In this context, Raman spectroscopy (RS) seems to be an ideal tool to investigate the quality of LPs, providing information on functional chemical groups present in the sample, quickly, virtually and without the need to target specific molecules [3].

The purpose of the present study is thus to test the RS's ability to provide information on the quality of the main classes of LPs: Very-Low-Density Lipoproteins, Low-Density Lipoproteins and High-Density Lipoproteins, extracted by ultracentrifugation in discontinuous density gradient from the plasma of obese patients (Ob, n=39) and healthy subjects (HC, n= 26).



Comparing the Raman spectra of Ob's LPs with those of HC, clear differences emerged in the composition between the classes of LPs and in their oxidative state. In particular, RS highlighted that Ob's LDL present lower amounts of carotenoids (1525 cm^{-1}) and unsaturated fatty acids (960 cm^{-1}, 1271 cm^{-1}, 1656 cm^{-1}) when compared with HC subjects. Ob's HDL were characterized by a depletion in the lipid components (cholesterol, 700 cm^{-1} and phospholipids, 717 cm^{-1}).

Overall, these results unravel the effectiveness of RS as a viable approach to gain information's on the quality of LPs, defined in their biochemical composition and oxidative state, and pave the road toward a more comprehensive understanding of the role of LPs in metabolic dysfunctions, where quality appears to be a better biomarker than the quantity to frame individual patient cardiovascular risk.

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OC09

Optimization of a molecular beacon-based signal-off SERS biosensor for miRNA detection

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With the advance in nanotechnology and metallic nanostructured materials, surface-enhanced Raman scattering (SERS) has opened exciting new routes for many biosensing applications for biomarker detection. Among all biomarkers, scientists are increasing their interest on microRNAs (miRNAs), which are small non-coding regulatory RNAs that have the potential to impact the development and progression of nearly all human diseases through interactions with mRNA. The strategy exploited in this work for miRNA detection is a signal-off mechanism by means of a labelled molecular beacon (MB) immobilized on a SERS substrate as the miRNA biorecognition element: a MB tagged with a Raman reporter was employed and the distance between the label and the SERS surface distinctly changed upon hybridization with miRNA, resulting in a large measurable SERS signal change.

The main objective of this work was the realization of a low-cost SERS substrate for in-liquid measurement of microliter sample volumes as well as the optimization of the metal/MB-analyte interface to achieve the highest sensitivity and reliability of the SERS signal-off MB biosensor. In particular, the MB for a miRNA specific for chronic obstructive pulmonary disease (COPD), was selected and designed.

For the specific recognition of the selected miRNA biomarker, miRNA-183, the design and synthesis of a MB, capable of changing its conformation when interacting with its specific target, was pursued. After characterization in fluorescence, the MB labelled with the Raman tag was exploited for miRNA SERS biosensing by using a multi-well liquid cell adapted for the SERS measurements. In order to maximize the SERS signal while ensuring the advantages of a cost-effective and practical assay, we developed a plasmonic platform based on a SERS substrate specifically designed to increase the local molecular density at the SERS hotspots produced from a network of silver nanowires (AgNWs).

Sub-femtomolar detection limits for miRNA-183 were obtained with this approach after optimization of the sensing surface. Good specificity and the possibility of performing multiple cycles of measurements after regeneration were also demonstrated.

Acknowledgements: We acknowledge the support of the European Union by the Next Generation EU project ECS00000017 'Ecosistema dell'Innovazione' Tuscany Health Ecosystem (THE, PNRR, Spoke 4: Nanotechnologies for diagnosis and therapy).

OC10

Turning CRISPR-Cas12a Activity ON Using PAM-Engineered DNA Translator Responsive to Antibody

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Integrating dynamic nucleic acid (NA)-based devices with protein-controlled actuation will expand our ability to process molecular information. Recently, we have developed different strategies to control synthetic switches and more complex reaction networks to convert specific protein-binding events into measurable outputs.[1,2] Here, we report on the rational design of an antibody-responsive DNA system able to trigger the activity of the CRISPR-associated (Cas) endonuclease enzyme. Specifically, we used Cas12a, a single-RNA-guided endonuclease that recognizes target double-stranded DNA (dsDNA) containing a complementary sequence to the crRNA guide along with a thymine-rich protospacer adjacent (PAM) site.[3] Our strategy employs a PAM-engineered DNA hairpin (i.e. Translator) and two synthetic antigen-labeled nucleic acid strands to create an artificial communication pathway between Cas12a proteins and target antibodies. Specifically, the Translator is designed so that the complementary portion of the PAM (PAM*) is hidden in the loop, thus making PAM not accessible to Cas12a. This trick prevents efficient recognition and efficient activation of Cas12a activity. In the presence of the specific target antibody, instead, the antibody-induced colocalization of the two antigen-labeled NA strands induces a conformational change to the Translator and PAM complementation. By doing so, Cas12a collateral nuclease activity is switched on, and an amplified fluorescence signal transduction is generated (Figure 1). Our single-step, modular sensing platform can be used to recognize any bivalent antibody for which an antigen can be conjugated to a nucleic acid strand. We demonstrate sensitive and specific detection of three antibodies in complex matrices (i.e. blood serum), including clinically relevant Ab as anti-HA and anti-MUC1 antibodies. We believe CRISPR-based diagnostics could extend the capabilities of clinically relevant diagnostic technology for the ultrasensitive and rapid sensing of biomarkers at the point of care.

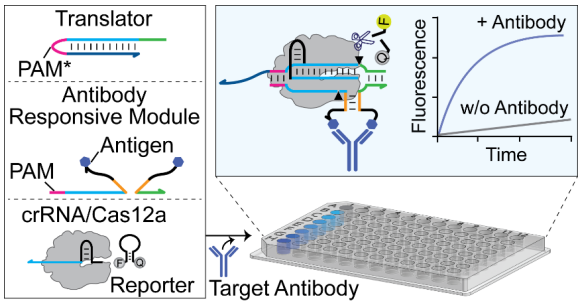

Figure 1. Turning Cas12a nuclease activity ON using antibodies.

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OC11

Design of Immunoassays based on a Plant-Produced Antibody

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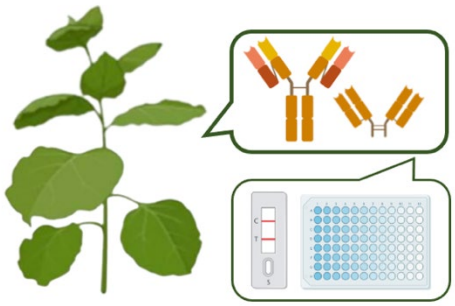
This work proposes the use of a new monoclonal antibody (mAb) specific to Aflatoxin M1 (AFM1) produced by the "Plant Molecular Farming" technique in collaboration with the ENEA Casaccia research center as an alternative diagnostic tool to the animal-based production of monoclonal antibodies. Immunodiagnostics offers numerous advantages and plays a crucial role in both biomedical and agri-food analysis sectors. The green biotechnologies offer new economically convenient solutions for the production, in engineered plant systems, of recombinant antibodies in large amounts. The antibody was obtained starting from the genetic information of a murine hybridoma, which was transferred to plants using the agro-infiltration technique. This involves the infiltration of a suspension of Agrobacterium tumefaciens containing the genetic information/script directly into the plant tissues, favoring the transfer of the DNA to a higher percentage of cells.



(1)

The new plant-derived mAb was involved in the development of two immunodiagnostic platforms for the detection of AFM1 in raw milk, an Enzyme Linked Immunosorbent Assay (ELISA) and a Lateral Flow ImmunoAssay (LFIA). These two assays had proven to be effective diagnostic tools for the quantification of AFM1 within the limits established by the law. (2)

The ELISA test has demonstrated good accuracy in the quantification range of 10-75 ng/L (CV% <25%). The total time required for test is 75 minutes, and the recovery rates from raw milk samples (88-104%) and from control materials (107%), confirmed the reliability of this diagnostic tool.

The LFIA device was established by comparing various porous materials and studying the assay reagents (e.g: the AFM1-BSA conjugate to be used as capture ligand) and format (selection of the mAb labelling strategy with gold nanoparticles: pre-absorption or addition in solution). The developed device allowed to observe the inhibition of the signal for AFM1 concentrations between 0.5 and 0.05 µg/kg in raw milk.

Recent developments in the production of proteins expressed by plants have led to the synthesis of mAb, the same used in the diagnostic tests described above, expressed linked to a fluorescent marker, the green fluorescent protein (GFP). The new fused protein has been investigated as a possible smart material to be incorporated into a fluorescent LFIA (F-LFIA). Preliminary evaluations of the properties through a typical LFIA strip and the stability to variation of temperature, pH and ionic strength and matrix composition have evidenced the feasibility of the mAb-GFP ligand for F-LFIA development.

The new antibody produced by plants can be a convenient substitute for monoclonal antibodies produced in vitro for the development of reliable assays, with advantages in terms of time and cost savings and their future use in simpler LFIA immunoassay platforms.

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OC12

Robust analytical performances of a label-free electrochemical biosensor for the detection of Interleukin-6

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Interleukin-6 (IL-6) is a soluble mediator with a pleiotropic effect on human health [1]. High concentrations of IL-6 are correlated with a wide range of diseases as well as in the hyper-inflammatory responses, autoimmune processes, and many illnesses such as diabetes, atherosclerosis, depression, and Alzheimer's [2-4].

Herein, the fabrication of an alternative novel label-free voltammetric immunosensor based on biocarbon nanomaterial (biochar) modified screen-printed electrodes (Bio-SPEs) for the rapid, low-cost, and accurate detection of IL-6 was presented. A carbodiimide-mediated amide coupling reaction (EDC/NHS) to immobilize the secondary antibody (Ab-IgG) onto biochar's carboxylic group and two different primary receptors, identified as mAb-IL-6 clone-5 and clone-7 antibodies, were used. The electrochemical characterization of the layer-by-layer assembly of the immunosensor was conducted by cyclic voltammetry (CV) and sensing was performed using square wave voltammetry (SWV). The two developed immunosensors, using clones-5 or 7 monoclonal antibodies, respectively, had good analytical performances in human serum, exhibiting a wide linear range (LR) from 12-205 and 15-238 pg/mL, a good limit of detection (LOD) of 8.8 and 10.4 pg/mL and selectivity for IL-6 over other common cytokines, including IL-1β and TNF-α. Performance comparison of IL-6 immunosensors with those of a spectrophotometric ELISA kit (LOD of 20 pg/mL) denoted a better sensitivity of the proposed label-free devices, associated with a reduced detection time (30 minutes instead of more than three hours for ELISA test). Furthermore, the successful application of the proposed immunosensors in blood samples (with only a dilution of 1:100 v/v in PBS and without additional treatments) with good sensitivity (LOD of 14.3 pg/mL and reproducibility, RSD% < 11%) paves the way for their application as a point-of-care viable alternative to the IL-6 detection techniques routinely used (ELISA and Western Blot). In conclusion, a version of a sandwich label-free device for the detection of the same analyte has been manufactured, and the preliminary results will be reported.

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OC13

Novel hydrogel films integrating Prussian Blue nanoparticles and enzymes: a versatile strategy for portable electrochemical (bio)sensing

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Hydrogels have emerged as promising soft porous materials with which to develop a wide range of biosensing platforms, due to the possibility of easily engineering their properties and effectively immobilising enzymes and nanomaterials in their matrix. Hydrogels prepared from renewable sources, such as cellulose derivatives, as biocompatible, biodegradable and capable of providing robust biosensor platforms while avoiding non-specific protein uptake from blood and biofluid samples, have attracted attention for biosensing applications.[1,2] Despite their interesting properties, the ability to form stable hydrogel films integrating catalytic nanomaterials and well-dispersed enzymes on electrode surfaces is still required to enable their implementation in electrochemical biosensors. The physico-chemical properties of hydrogels can be easily adapted because the cross-linked network can be effectively used to trap enzymes and nanoparticles (NPs), which give the hydrogel plasmonic, catalytic or conductive properties. Prussian blue (PB) NPs are one of the most versatile and efficient catalytic nanomaterials to be applied to sensors and biosensors.[3] In the present work, a versatile strategy is presented to prepare hydrogel films incorporating Prussian blue nanoparticles (PBNPs) and different enzymes on the electrode surfaces for electrochemical biosensing. A one-pot strategy was employed for the synthesis, under mild conditions, of the PBNPs and the simultaneous immobilisation of the enzymes in a highly functionalised carboxymethylcellulose matrix, resulting in homogeneous hydrogel composites. Composite hydrogels have been applied in combination with screen-printed electrodes for the development of miniaturised devices for the detection of species relevant for biomedical applications, in particular glucose and NADH. Two amperometric biosensors for the rapid detection of glucose and ethanol in serum were realised using hydrogel composites integrating glucose oxidase and alcohol dehydrogenase. In standard solution, a LOD and LOQ of 7µM and 20µM for NADH and 50µM and 200µM for H2O2 were obtained. To evaluate the effectiveness of the system in a biological matrix, serum samples were used, obtaining good results in terms of sensitivity. By combining the unique features of hydrogels on flexible electrochemical strips, our approach holds great promise for the development of portable, easy-to-manufacture and versatile electrochemical (bio)sensors for the detection of a variety of analytes.

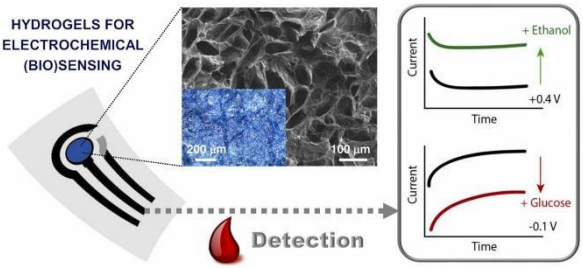

Figure: Principle of operation of the glucose and ethanol biosensor

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OC14

In Vitro Inhibitory Effect of Green Tea Catechin Extracts against the SARS-CoV-2 Papain-like Protease Activity

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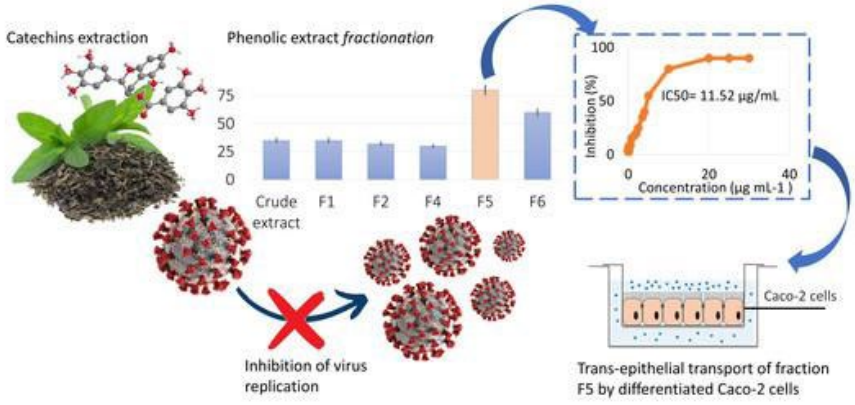
COVID-19 is a viral illness caused by the SARS-CoV-2 virus, which is responsible for the current pandemic outbreak. The pandemic emergency prompted the scientific community to pursue efforts for developing pharmacological therapies through the screening of some old drugs capable of working against SARS-CoV-2 and vaccines for counteracting this new disease threat [1]. In this context, identifying natural compounds able to prevent infection represents an efficient and complementary strategy. Indeed, many bioactive substances, which are naturally present in foods, have widely displayed potent biological activity [2]. Therefore, natural substances, especially those within plant-derived phytocomplexes, may represent sources of active compounds that may synergistically impair the SARS-CoV-2 infection and COVID-19 progression [3]. Catechins, a class of phenolic compounds, primarily present in food products, such as cocoa, red wine, fruits, vegetables, and tea leaves, are already well known for their interesting health-promoting activities, including anti-inflammatory and antioxidant antibacterial, anticancer, and neuroprotective ones [4]. In this field, green tea (*C. sinensis*) is one of the wealthiest catechin sources that contains (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin-3-gallate (EGCG) as its major catechin components. This work [5] describes an untargeted analytical approach for the screening, identification, and characterization of the trans-epithelial transport of green tea (*Camellia sinensis*) catechin extracts with in vitro inhibitory effect against the SARS-CoV-2 papain-like protease (PLpro) activity. After specific catechin extraction, a chromatographic separation, based on reversed-phase liquid chromatography (RP-LC) was carried out for obtaining six fractions (F1-F6) which inhibitory activity was assessed in vitro against the PLpro target. The fraction with the highest inhibitory activity against the SARS-CoV-2 PLpro (F5 with IC_{50} of 0.125 μg mL^{-1}) was further analyzed by high-resolution mass spectrometry (HRMS) to annotate its composition. Since little is known about the absorption and interaction of catechins within a phytocomplex whose complex composition may interact and modulate the transport, using Caco-2 cells as a model, the potential for intestinal transportation of relevant catechins was investigated. The untargeted characterization revealed that (−)-epicatechin-3-gallate (ECG) was the most abundant compound in the fraction and the primary molecule absorbed by differentiated Caco-2 cells. Results indicated that F5 was approximately 10 timesff more active than ECG (IC_{50} value equal to 11.62 ± 0.47 μg mL^{-1}) to inhibit the PLpro target. Overall, our findings highlight the synergistic effects of the various components of the crude extract compared to isolated ECG.



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OC15

An *origami* paper-based chemiluminescent biosensor exploiting a structure-switching ATP-binding DNA aptamer

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Microfluidic paper-based analytical devices (μPADs) have attracted considerable interest in recent years for the development of rapid and inexpensive point-of-care (POC) diagnostic tests to help improve global public health. Several μPADs have been proposed for a range of target analytes, exploiting various biorecognition and transduction principles (1). Enzyme immunoassays are the gold standard for the specific detection of a wide range of target analytes of diagnostic interest in complex samples. However, the limited stability of antibodies and enzymes limits their POC applicability. Target-responsive DNA-based nanomaterials have attracted substantial research interests in the fields of disease diagnostics, exploiting their ability to provide both target biorecognition and signal generation (2). Coupling functional DNA-based biosensors with μPAD format thus appears to provide the features for successful POC applicability (3). However, ensuring proper functional DNA binding and folding on the paper support is still a challenging task and, in many cases, most of the assay protocol is performed in solution, only dedicating the μPAD to the final signal readout.

We recently developed a structure-switching ATP-binding DNA nanoswitch with two functional domains: a catalytic DNA-zyme domain and an ATP-binding aptamer domain (4). In the presence of ATP, its binding to the aptamer domain triggers the activation of the DNA-zyme domain, which is exploited for chemiluminescence detection. In this work, we present the development of an origami μPAD in which all the reagents are preloaded on the origami layers in the dried form and the whole assay procedure is conducted on paper upon simple addition of sample and a buffer solution and proper folding and unfolding of the paper layers. Paper functionalization strategies and assay protocol were optimized to ensure simple and straightforward detection of ATP, selected as a model analyte, in the *origami* μPAD format, employing a portable charge-coupled device (CCD) camera for chemiluminescence detection.

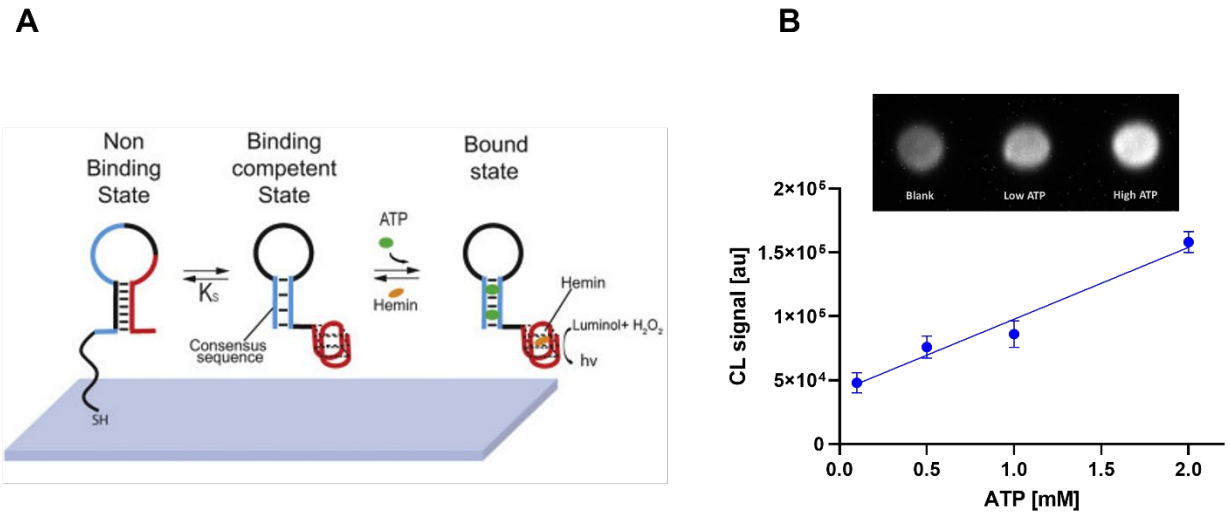

Figure1. (Left) ATP-regulated DNA-nanoswitches mechanism. (Right) ATP calibration curve by chemiluminescent imaging.

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OC16

Estradiol detection in water samples related to aquaculture exploiting plasmonic spoon-shaped biosensors

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A polymeric multimode waveguide, characterized by a pioneering spoon-shaped geometry and featuring a built-in measuring cell [1], was herein proposed to devise a Surface Plasmon Resonance (SPR) platform to monitor the specific receptor-analyte interaction for estradiol identification in water samples.

The fabrication process to realize the biosensor consists of two steps. At first, a gold deposition on the spoon-shaped waveguide surface was realized through a sputter coater machine to obtain the SPR platform. Then, the functionalization of the SPR sensitive surface with a specific estrogen receptor (αER), able to recognize the estradiol, was performed through an immobilization process of the receptor to a Self-Assembled Monolayer (SAM) produced on the gold surface.

The biosensor response performances were tested in physiological conditions (phosphate buffer, 10 mM, pH 7.4) and in different real water matrices related to fish aquaculture (freshwater and seawater).

The estradiol concentration was monitored in all matrices between 360 fM – 36 nM. A limit of detection (LOD) equal to about 0.1 pM was obtained for all the explained conditions. The proposed biosensor experimentally tested showed a high sensitivity for the detection of low concentrations of analyte, and the comparison between the parameters obtained for the measurements in different matrices demonstrated that the matrix variation did not significantly influence the platform's response.

As a result, the experimental outcomes supported the possibility of using the αER-based biosensor for the quantitative determination of estradiol in different real water samples (freshwater and seawater) in the range of concentration requested by a real scenario, without any sample pre-treatment (i.e. preconcentration or extraction).

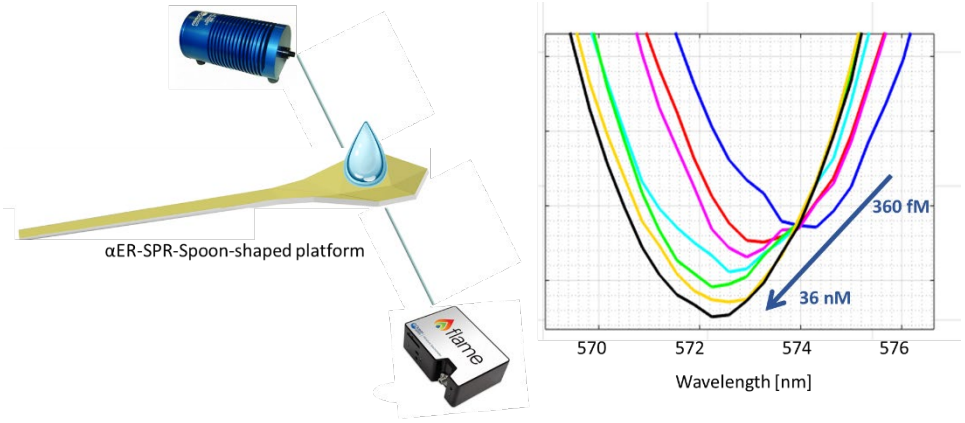

Figure 1: Outline of experimental setup adopted to test the SPR-spoon-shaped biosensor and SPR spectra relative to estradiol detection at different concentrations ranging from 360 fM and 36 nM.

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OC17

Smartphone-based colorimetric paper sensor for food quality control based on biogenic amines monitoring

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One of the main causes of food spoilage is the growth of microorganisms originally present in the food or arising from external contaminations because of inadequate storage conditions or food processing. Biogenic Amines (BAs) are nitrogenous thermoresistant molecules naturally occurring at low concentrations in different protein-rich foods, and their amount increases during food spoilage. According to the European Food Safety Authority (EFSA), putrescine and cadaverine are the most common BAs found in food [1].

Several analytical techniques for the detection of BAs have been reported, but they need sophisticated instrumentation and skilled personnel. In this work, we present an inexpensive colorimetric paper sensor for quantitative smartphone-based detection of BAs, using small sample volumes [2]. The sensing molecule is the aglycone genipin, a natural cross-linking agent extracted from gardenia fruit, able to bind BAs producing water-soluble blue pigments. A T-shaped sensing paper was designed and printed on paper using a wax-printer, providing a disposable device for detecting BAs in food samples, easily readable by consumers. The colorimetric BA sensing paper consisted of three circular wells with a diameter of 5 mm, two of which were used as chromatic indicators of BA presence (HARMFUL well) or absence (SAFE well). The third well was the sample well for detecting BAs developed in real samples, where genipin was entrapped. Colorimetric images were acquired with a OnePlus 6 smartphone, and reflectance signals were evaluated by brightness analysis with ImageJ software. The system was tested on chicken meat samples by integrating the sensor into smart packaging for qualitative and quantitative BAs analysis based on a smartphone detection, achieving a limit of detection of 0.1 mM of putrescine (Figure1).

With increasing demand to monitor food quality, this sensor represents a green and user-friendly platform both for food industry and consumers. It can be integrated into smart packaging for rapid food freshness and spoilage evaluation by necked eye.

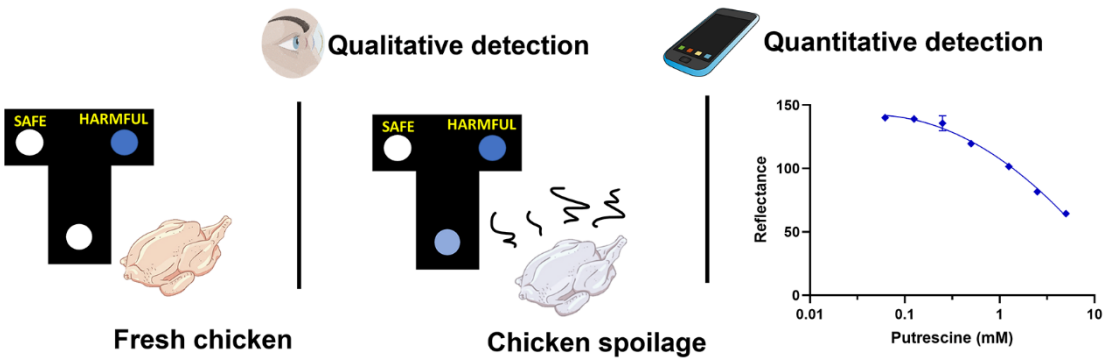

Figure 1: Schematic representation of the colorimetric paper sensor for food spoilage based on BAs monitoring

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Acknowledgements We acknowledge Ph.D. programs on green topics (PON "Research and Innovation" 2014–2020) funded by FSE REACT-EU, and the FEDKITO project funded by MIUR and Prima (Partnership for Research and Innovation in the Mediterranean Area).

OC18

From one-pot to tailor-made: an FFF-assisted analytical approach to develop high quality and ready-to-use conjugated gold nanoparticles for sensing applications.

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Gold nanoparticles (AuNP) are a benchmark in lateral flow immunoassay (LFIA) development given their versatility as a transducer and their ability to change colour in a reagent-less mode. The main parameters affecting AuNP properties and performance are their size and size distribution, directly influencing their plasmon resonance band, and their stability after their modifications (such as coating, antibody conjugation, labelling). In order to develop efficient, high quality nanoparticles for biosensing, it is necessary to achieve reproducible conjugation stoichiometry (affecting sensitivity), purify size-uniform conjugates, and verify that they are stable in the intended application, where salinity and pH differ from the synthesis batch. Conjugation and capping of AuNP is usually done in cycles of mixing and washing through centrifugation, which is also the preferred purification step. Conventional QC steps involve the use of batch UV spectrophotometry and dynamic light scattering (DLS), that yield however only averaged results. Both of these steps can be improved dramatically with the use of field flow fractionation-multidetection (FFF-MD), a separation and characterization technique able to i. size separate bound and unbound conjugants, ii. monitor the conjugation efficiency and recover unreacted labels, iii. Characterize AuNP in terms of size, shape and spectroscopic properties, iv. Evaluate stability and behaviour in different media and native conditions, and last v. Isolate purified, stable particles for further use in biosensing. The work presented will illustrate how FFF-MD proves useful in collecting both information and ready-to-use AuNP, in a short analysis time and scalable approach, and how FFF-MD can also be a really attractive technique to study AuNP conjugation mechanisms to identify the yield-maximizing and product-maximizing conditions for the generation of new AuNPs conjugates.

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OC19

Improving sensitivity and versatility of lateral flow immunoassays maintaining the full compliance to ASSURED criteria

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The importance of Point-Of-Care Tests (POCTs) as intrinsically strategic analytical tools has been highlighted by the recent SARS-CoV-2 pandemic [1]. Considering its impact on the society, the research focuses the efforts on improving performances (sensitivity, sustainability, miniaturization, etc...). Despite POCTs are meant to be compliant to ASSURED requirements (Affordable, Sensitive, Specific, User friendly, Rapid-robust, Equipment-free, Deliverable) by the WHO [2], many papers describe devices based on novel materials, reagents, high-tech readers, or sophisticated technology, that hardly fulfil these criteria, being scarcely appliable on-field, and/or affordable for everyone. The case of the lateral flow immunoassays (LFIA), stand-alone rapid POCTs, based on the antibody-antigen specific interaction, is a striking example of how the simplicity and affordability represent a key factor for the application for benefit the community [3]. The LFIA, for instance, is widely employed in diagnostics for its total compliance with the ASSURED criteria. The typical LFIA strip is a portable device composed by overlayered materials containing all the reagents required for the assay, meant to give response in few minutes in reactive areas, called test (T) and control (C) lines. The current gold standards are represented by red-coloured gold nanoparticles as the labels, antibodies/antigens/ligands as capture (CIR) and detection (DIR) immunoreagents, glass fibre/cellulose-based materials, and visual readout. The main reported drawback of typical visual gold based LFIA is the low sensitivity, hence the perceived need of novel materials, detection systems, or technologies. My work at the Department of Chemistry of the University of Turin focuses on a deeper understanding of the nature of the involved phenomena can effectively improve the performance without affecting compliance with ASSURED criteria, in diagnosis of SARS-CoV-2, HIV, Foot-and-mouth disease, Lumpy Skin Disease, and African Swine Fever Disease. Some examples, in the field of serological testing, are: i) the total antibody detection approach employing aiming to specific antibodies by using recombinant viral antigens both as CIR and as DIR, avoiding the saturation of the random antibodies from the serum that generally affects typical antibody tests, including other antibody classes (IgA and IgM) in the detection [4 and other unpublished observations]; ii) the use of bacterial ligands, in particular, staphylococcal protein A (a high-capacity antibody ligand with 5 binding domains), as the labelled reagent, to increase capacity and so the sensitivity [5]; iii) the study on how the sensitivity changes by using the same immunoreagents as CIR or DIR, and how this enable multimodal-multiplex detection [6]. Concerning antigen detection: iv) important understanding was achieved by exploring the most influent variables by means of experimental design [7] and v) an opposite behaviour is revealed if the CIR and the DIR are the same immunoreagent (single epitope, SE, sandwich), or not (double epitope, DE, sandwich), and a model for the explanation and interpretation was proposed [8]. All these findings improved considerably our knowledge of LFIA technique and allowed us to find strategies to keep the simplicity of the visual LFIAs without renouncing to sensitivity. This is crucial if we want to keep the POCT available where there are not the circumstances for novel (but inaccessible) devices.

OC20

Metal nanoparticles laser patterning for cellulosic devices development

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The use of metal nanoparticle (MNP) based devices has become common (e.g. lateral-flow immunoassay), thus the search for new strategies to integrate these nanomaterials in flexible substrates and devices is a hot topic. In this framework, emerging approaches for in-situ nanodecoration and paper-based devices-manufacturing employing bench-top equipment, are more and more required because of their low-cost and high analytical potential [1]. Particularly, the direct MNPs decoration onto flexible supports (i.e., cellulosic, flexible plastics, textiles, etc.) can allow the implementation of new (bio)sensors and (bio)sensing strategies.

In this presentation, a fast CO_2 laser writing strategy to in-situ synthesize MNPs with high-resolution patterns (up to the micrometric domain) onto cellulosic substrates will be presented. In brief, the CO_2 laser plotter induces the formation of MNPs according to a previously patterned design, resulting in MNPs with controlled size embedded in the cellulosic structure. The strategy proposed allows the MNP decoration of cellulosic, polymer, plastic, and conductive substrates [2]. Among others, this approach has been used to develop MNPs composed of Au, Ag, Pt, Ni, and Cu onto paper sheets (Figure 1); the nanostructured surfaces have been employed to realize different paper-based devices.

The nanostructured paper has been employed to build up a device for the real-time colorimetric monitoring of oxidative vapors (i.e., peracetic acid) commonly used for cleaning surfaces in industrial, public, and healthcare areas. In this case, the MNPs sensing array, composed of AuNPs, AgNPs, PtNPs, NiNPs, and CuNPs exploits the different nanoparticle features and chemistry, allowing the peracetic acid monitoring at exposure levels used in disinfection treatments. The same paper-based MNPs-array was used also for the remediation of commonly used organic dyes (i.e., methyl orange, methylene blue, and rhodamine B), proving the catalytic features of the laser nano decorated-paper sheets.

In addition, a sensing strategy for the monitoring of reducing biomolecules (i.e., L-cysteine, GSH, ascorbic acid, etc.) has been also proposed; in this case, was exploited the catalytic ability of the PtNPs decorated paper surface, that allows the colorimetric conversion of tetramethylbenzidine (TMB). Exploiting this feature, a paper-based colorimetric device for the reagent-free determination of biocompounds was proposed; the application of the device toward various food and biological samples is in progress.

All the paper-based devices proposed have given rise to analytical strategies for which is required just a smartphone to obtain satisfactory analytical figures, resulting in being user-friendly and affordable. Summing up, the here proposed CO_2-induced nanodecoration strategy represents an appealing opportunity for the development of a new generation of analytical devices based on custom-designed nanoparticles.

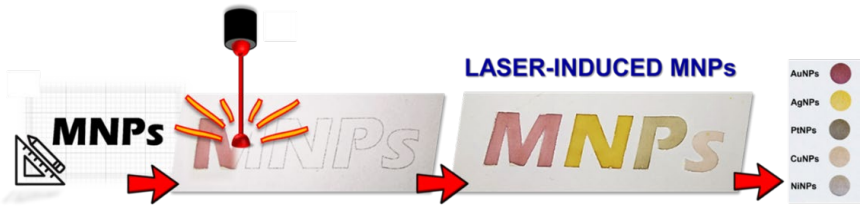

Figure 1. Sketch of the laser-induced MNP formation and pictures of the obtained nanodecored paper-surfaces.

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This research was funded by the European Union – Next Generation EU. Project Code: ECS00000041; Project CUP: C43C22000380007; Project Title: Innovation, digitalization and sustainability for the diffused economy in Central Italy - VITALITY

OC21

Nanostructured electrochemical platforms: building biomimetic sensors for food applications

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The presence of harmful chemicals and microorganisms in food is often addressed ad food contamination, which can cause consumer illness at different levels of severity. Aptasensors can become a helpful tool in the detection of contaminants and allergens as a highly sensitive and easy-to-use analytical procedure. Moreover, the introduction of nanostructured systems allows the miniaturization and the portability of the devices, which make them suitable for on-field screening analysis. The aim of this presentation is to give an overview on the most recent important achievements of our research group in the realization of nanostructured electrochemical platforms based on conducting polymers and noble metals nanoparticles and their successful application in the detection of molecules of clinical interest and/or in the development of DNA-based biosensors to be applied in food analysis. The developed sensing platforms can be exploited, indeed, for an easy and rapid determination of electroactive analytes or further implemented and used as a scaffold for bioanalytical assays built on different formats. Specifically, three different platforms are presented: the first one is composed of the copolymer poly(aniline-*co*- anthranilic acid) and was applied for a competitive assay detecting the mycotoxin aflatoxin B_1 (AFB1) [1]; the second one is a nanocomposite built up with polyaniline and gold nanoparticles to detect the mycotoxin deoxynivalenol (DON) with a competitive assay [2]; the third one is a nanocomposite similar to the second one but that sees the replacement of polyaniline with poly-L-lysine to detect the milk allergen *β*-lactoglobulin by means of a switch-on assay [3]. This overview underlines the possibility of using and applying structurally similar platforms in different architectures, which results outstanding in the perspective of a low-cost and multianalyte research work, to allow the development of robust devices to be successfully applied in several fields of analysis.

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OC22

Solid-phase synthesis of nanoMIPs: how the parameters optimization can improve their binding ability

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The solid phase polymerization synthesis (SPPS) is a versatile and innovative approach to obtain molecularly imprinted nanopolymers with very high affinity and selectivity for the target molecules [1]. In this technique the template molecules are not free in the polymerization medium, but they are immobilized onto the surface of a solid support, usually glass beads. The polymerization process takes place in the interstitial space between non-porous glass beads grafted with template molecules. Once the polymerization process ends, unreacted monomers, polymerization by-products and low-affinity polymers can be washed away, while the high-affinity nanoMIPs bind strongly enough to be retained by the solid phase. NanoMIPs are subsequently eluted by washing the solid phase with a solution capable of breaking the non-covalent molecular interactions. After the polymerization, nanoparticles are obtained with hydrodynamic diameters of 120/150 nm and with imprinted binding sites only on the surface and not in the bulk of the polymeric structure.

This approach presents several practical advantages over traditional approach: the bleeding effect due to residual template molecules in the imprinted polymer is avoided, grafted templates do not need to be soluble in the polymerization solvent, thus eliminating any issue about solvent-template compatibility. The solid phase can be reused many times, allowing the use of expensive molecule, toxic or harmful templates. The confinement on the surface of the beads of this type of templates eliminates any health risks from residual template during the recover step of the imprinted nanoMIPs.

Here we present the results concerning the optimization of SPPS parameters. First of all, it was considered an experimental design on the formulation of ciprofloxacin-imprinted nanoMIPs, changing the molar ratio of the monomers, and evaluating how the binding proprieties – affinity and selectivity – vary accordingly [2]. Then, on the basis of the best polymerization mixture, it was studied the influence of the polymerization time – ranging from 15 min to 3 hours – on the same proprieties. The binding properties were studied by partition equilibrium and rebind kinetic experiments to measure the binding affinity (K_{eq}) and the kinetic rate constants (k_d, k_a). Furthermore, selectivity and non-specific binding were evaluated by measuring the rebinding of levofloxacin onto ciprofloxacin-imprinted nanoMIPs, and ciprofloxacin onto diclofenac-imprinted nanoMIPs, respectively. Intermediate times (45-60 min) produced nanoMIPs with high affinity ($>10^6$ M^{-1}) and good selectivity [3]. Finally, it was studied the influence of the crosslinker on the binding proprieties and the shape of nanoMIPs imprinted against rabbit γ-globulins. The possibility to obtain MIP imprinted for the protein is a long-time goal, and with the SPPS technique it has become easly feasible. Five crosslinkers with different polarity and rigidity were considered, and for each one the binding proprieties were measured. The obtained results indicate that some type of crosslinker give better binding proprieties to the nanoMIPs, but their effect on the morphology and dimension is insignificant [4].

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OC23

Thermogravimetry coupled with chemometrics for the differential diagnosis of hereditary hemolytic disorders

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The coupling of thermogravimetry and chemometrics proved to be a fast and reliable analytical tool for hematological investigation [1-3]. In this study, a multi-screening test for the differential diagnosis of hereditary hemolytic anemia was developed [4]. Thermogravimetry (TG) was used to analyze whole blood samples from patients affected by thalassemia, sickle cell anemia, hereditary elliptocytosis and hereditary spherocytosis as well as samples collected from healthy donors. Thermal analysis permitted to highlight a specific hemolytic state, caused by defects on hemoglobin or membrane of red blood cells, as a characteristic thermal behavior was obtained for each considered congenital disorder. Chemometrics permitted a multiparametric evaluation of the recorded TG curves ensuring a time-saving analysis. The Partial Least Squares - Discriminant Analysis (PLS-DA) was the selected chemometric technique for the validation of an accurate and precise prediction model. The TG/Chemometric method permitted to perform an effective first level diagnosis in about 1 hour using only 30 µL of blood non-pretreated. All samples involved in the study were analyzed by the reference method required for the first level diagnosis and the confirmatory analysis confirming the feasibility of the novel multi-screening test.

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Presentazioni Flash

PF01

Exosomes and their Cargo as "Two In One" Liquid Biopsy Tools for Cancer Biomarkers: New generation of Luminescence-Based Biosensors Integrated with FFF Isolation From Biological Fluids

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Early-stage diagnosis of cancer is a crucial clinical need and new specific biomarkers are continuously searched by metabolomics and proteomics studies. The inadequacies of surgery tissue biopsy have prompted a transition to less invasive molecular profiling of biofluids known as 'liquid biopsy'. Exosomes (Exo) are phospholipid bilayers vesicles–having a biologically active cargo, and they perform cell-to-cell communication functions in biological systems. They are present in many biofluids i.e., plasma and serum, urine, semen, amniotic and cerebrospinal fluids. An increase in Exo excretion and their "cargos" are potential diagnostic biomarkers for an array of diseases, including cancer. Cancer cells release significantly more Exo than normal cells, containing a complex and heterogenous class of compounds. Therefore, the main analytical challenge is to develop a biosensor able to detect Exo concentration and simultaneously analyze specific biomarkers contained in their cargo. The developed device include on-line a preanalytical step able to enrich Exo from plasma or other body fluids allowing the biosensor to act on a concentrated, simplified and homogeneous fraction. Exosome fraction have been isolated with Field flow fractionation (FFF) using a miniaturized hollow-fiber able to collect, without relevant dilution, the needed Exo fraction according to their size (30 - 200 nm) and morphology. The selected fraction is introduced in a microfluidic assisted biosensor device. Exo are detected via their membrane specific CD63 protein with a capture rabbit-Ab anti-CD63 antibody followed by the addition of secondary Ab labeled with a Lanthanide chelate (1). The luminescence signal is measured in time resolved delayed fluorescence mode (TRF) to minimize the interference of sample autofluorescence. A miniaturized portable TRF instrumentation was used. The biomarkers will be quantified by thermochemiluminescence (TCL) TRF mode. The analyte specific Ab in immobilized on ITO support and after a stop flow of 5 minutes the secondary Ab labeled with a TCL probe (2) is added and after a washing step the ITO support brought at 120 °C allowing to decompose the TCL probe with emission of light which is recorded with a thermally cooled BI-CCD camera. Among luminescence detections TCL and TRF are reagent-less and do not need any reagent to trigger the luminescence thus facilitating the miniaturization and the microchip format using the same detector. Actually, TRF presents more performant biosensing peculiarity and two Lanthanide ion i.e. Tb^{3+} and Eu^{3+} can be used to achieve light emission at two different wavelengths (1). TCL signal is weaker but with a high signal-noise ratio and biomarkers can be detected down to pM levels. The limit of detection of Exo was as low as 5.10^2 Exo particles/µL. Although Exo could be considered a noninvasive "liquid biopsy" with great potential in the early diagnosis of cancer, Exo detection is still challenging: Some advanced devices have been achieved in the present work, which outlines the applicability of this approach. However, the main shortcomings including isolation and structure modification and detectability limits still hinder their applications, asserting the need for further optimization mainly in the FFF biosensor coupling.

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PF02

IMMUNE-CRISPR: combining immunoassays with Cas12a based detection using antibody-DNA conjugates

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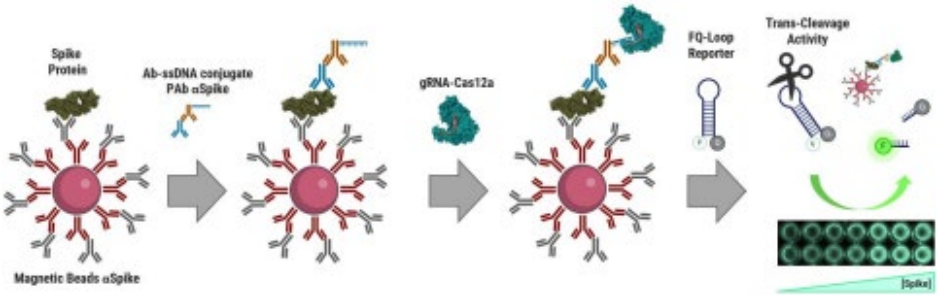
CRISPR-Cas (i.e., Clusters of Regularly Interspaced Short Palindromic Repeats) technology has been widely applied in the field of genome editing and, more recently, in molecular diagnostics of nucleic acids. Particularly, CRISPR Cas12a-based methods rely on the association of Cas12a enzyme with a guide RNA sequence (gRNA) to form a ribonucleoprotein (RNP) complex capable of recognizing a specific double stranded or single stranded DNA target sequence. In the case of single stranded DNA target, gRNA has to be simply complementary to the target molecule to generate a measurable and amplified output. Specifically, the target recognition by the RNP complex induces a site-specific nuclease activity on the target itself. Simultaneously, the recognition event induces a secondary "collateral" nonspecific nuclease activity (i.e., *trans*-cleavage activity) that is able to non-specifically digest all single-stranded DNA oligonucleotides present in solution[1]. In presence of single strand DNA modified with a fluorophore-quencher pair at the 5'- and 3'- ends (i.e., DNA reporter), the collateral nuclease activity cleaves the reporter producing a fluorescence increase due to the change of the distance between fluorophore and quencher pair. This process has been widely exploited as an amplified transduction mechanism for diagnostic application[2]. In this work we have combined CRISPR-Cas12-based detection system with a sandwich immune assay to achieve detection ofprotein targets, demonstrating the possibility to combine CRISPR technology with standard antibody

based assays. As a proof-of-concept demonstration, we designed a sensingplatform based on an immuno-magnetic assay[3] that uses CRISPR-Cas12a mediated signal transduction for the detection of SARS-CoV2 infection (i.e., Spike protein). In our approach, we conjugated the Cas12a activating DNA strand to anti-IgG antibody that is associated to the last recognition event of the immunoassay. By doing so, in the presence of the Spike protein, the antibody

DNA conjugate is recognized by the RNP complex through Watson-Crick interactions and this event triggers the nonspecific nuclease activity of the Cas12a enzyme. To improve detection performance of the system we used a more sensitive DNA reporter based on hairpin-type secondary structure (Figure 1) that enhances the system's response time and signal gain[4]. The optimized assay proved capable of detection SARS-CoV-2 associated Spike protein achieving high specificity and good analytical sensitivity (LOD=15 ng/ml). Further investigations will be conducted to assess the matrix effect by testing the assay in biological samples such as saliva.



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PF03

NanoMIPs for thyroid hormones: an enantioselective challenge

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The human thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), both exist in two enantiomeric forms: Levo and Destro. The Levo gives the principal active effect through biological pathways while the D isomer is a by-product of the LT_4 synthesis. L-thyroxine has been selected as a template to explore the potential chiral recognition capability of nanoMIPs. In this work it is proposed a different strategy of polymerization respect to the classical bulk approach: the solid phase polymerization synthesis (SPPS), which shows numerous advantages in terms of homogeneity of binding size, no bleeding phenomena, protocol flexibility and high surface/mass ratio [1].

The L-thyroxine molecule presents a chiral center and two different kinds of function through which is possible immobilized the LT_4 onto the silica beads: the amino group and the phenolic group. Two different nanoMIPs have been synthesized through the solid phase polymerization synthesis method, where the orientation of the template covalently linked to the silica beads gives the difference between the two. The "direct" – (d)nanoMIP – was prepared by covalent grafting to hemisuccinated silica of LT_4 directly through the amino group. In this case, the binding takes place from the proximal part to the chiral center and there are two function available for the recognition effect. The "reverse" – (r)nanoMIP – was obtained by grafting onto aminated silica the template on the phenolic group side, in the farthest part of the molecule respect to the chiral center. In this case, there are three function available for the recognition effect. To prepare this template, LT_4 was modified with a carboxymethylether group linked to the phenolic function (L-T_4 CME). NanoMIPs were covalently linked to silica beads and batch rebinding experiments were performed with different ligands (LT_4, DT_4, LT_3, DT_3 and LT_2) to obtain the corresponding binding isotherms and, thus, evaluate binding affinity, selectivity and enantioselectivity.

It was found that (r)nanoMIP shows the higher affinity for the template LT_4, with a binding constant of 1.08×10^8 M^{-1}, which is fourfold higher than the binding constant for (d)nanoMIP. This increase of affinity could be related to the oriatation of the template molecule and the avaibility of more functional groups to the interaction with the polymer. Concerning selectivity, both the nanoMIPs show a clearly decreasing affinity for the structural analogs with less iodine substituents. In particular, (r)nanoMIP shows a remarkably selectivity not only towards the different thyroid hormones, but also between the different enantiomeric forms of T_4. Consequently, it efficiently discriminates the L-enantiomer from the D-enatiomer and, therefore, it recognizes the template with very high selectivity. In conclusion, the imprinting against the inverted template confirms that to obtain enantioselectivity the chiral center must be exposed to the growing polymer, far from the covalent link to the solid support.

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PF04

Set up and optimization of a LC/MS method for the characterization and control of mAbs Part 1: selection of stationary phase and digestion protocol

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Progress with recombinant DNA technologies has paved the way to producing recombinant proteins that can be used as therapeutics, vaccines, and diagnostic reagents. These recombinant proteins are complex macromolecules expressed by living cells and displaying intrinsically high micro-heterogeneity due to chemical and enzymatic modifications that can readily occur from the production to the storage phase, making the development of analytical methods for their characterization a difficult task. The heterogeneity could lead to numerous possible conformations, undesired Post-Translational Modifications (PTMs) and degradation, which could affect physical, chemical and biological proprieties [1], influencing, therefore, the product safety and efficacy profile. The main variability sources may include size, charge, oxidation, deamidation and N-glycosylation. Thus, extensive analysis and characterization are required even at an early stage of drug development to establish a well-characterized molecule and a good understanding of the protein structure, activity, immunochemical and physicochemical properties, purity, impurities and the impact of process changes on these attributes. [1] More recently, Multi-Attribute Method [2] (MAM) - a bottom-up approach where the protein is enzymatically digested and the obtained peptides are LC-MS analysed (peptide mapping) - has been proposed as promising approach for Quality Control (QC) of therapeutic proteins. In fact, it can measure multiple Product Quality Attributes (PQAs) in the context of drug development and characterization. This approach could be a powerful tool also to monitor Critical Quality Attributes (CQAs) at release and during the product lifecycle, decreasing the number of assays used and potentially reducing costs. Monoclonal antibodies (mAbs) remain one of the most important class of biotherapeutics in development. The diversity and complexity of mAb variant structures as well as the increasing emergence of biosimilar mAbs can create significant analytical challenges. For this reason, the aim of the work is to optimize a peptide mapping protocol for mAbs samples, to be applied in future to further develop a specific MAM for the characterization and QC of biopharmaceutical drugs, both in a manufacture and a regulatory environment, to help guaranteeing their quality, stability, safety, and efficacy. [3] When developing a reproducible and informative peptide map, the enzymatic digestion protocol and the separation of the resultant peptides need to be optimized. Thus, the first objective was to screen the performance of different chromatographic stationary phases in a LC/MS setting. In fact, poor chromatographic separations can result in rework and even compromise the accuracy of mAb characterization. To this purpose, Infliximab was used as a model for the biopharmaceutical class of mAbs, given its extensive and well-known characterization in terms of sequence and PQAs. Different tryptic digestion protocols were applied, and the stationary phases selectivity towards the generated peptide populations was considered. The digestion protocol efficiency was evaluated by comparing the performance of enzymes with different purity grade, at different time [4] and temperature conditions for the incubation phase. The impact of these factors on the overall quality of the peptide map was investigated taking into account the number of peaks detected, the total amino acidic coverage, as well as the number of missed- and non-specific cleavages.

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PF05

Second-generation blood microsampling for LC-MS/MS analysis of THC, CBD and their metabolites

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In the last decade, bioanalytical scientists have focused great efforts on the design and development of innovative volumetric microsampling technologies able to overcome some of the drawbacks inherent to dried blood spot (DBS) microsampling. These new approaches consist of capillary- or microfluidic-based biosample collection able to obtain volumetrically accurate miniaturised samples independently of density (and haematocrit in case of whole blood) in a minimally invasive manner, to be stored and transported in dried form without the need for temperature-controlled conditions (e.g. dry ice and ultra-freezers)[1]. Such kind of microsampling is now becoming mature for widespread application as viable and feasible alternatives to classic collection of "in-tube" fluid samples, which in turn are characterised by high invasiveness, expensiveness, reagent and solvent-demanding protocols, logistical drawbacks and stability issues. Novel microsampling tools for whole blood analysis provide simplified and reliable procedures for the collection of fixed and accurate volumes of whole blood directly by fingerprick. For this reason, these strategies could also pave the way to self- and home-sampling by subjects and patients themselves, towards the concept of personalised and remote medicine.

In this study, second-generation whole blood microsampling approaches based on capillary and microfluidic technologies were developed for the analysis of two major phytocannabinoids (namely Δ9-tetrahydrocannabinol – THC, and cannabidiol – CBD) together with their endogenous metabolites.

THC-type *Cannabis Sativa* L. is still considered illegal in several countries due to its psychotropic effects, yet scientific research has shown that some of constituents of both THC- and CBD-type *C. Sativa* may have significant therapeutic importance in the treatment of some diseases[2]. Thus, the development of innovative analytical platforms able to provide a reliable quali-quantitative assessment of THC, CBD and their relevant metabolites can be critical in several bioanalytical contexts, e.g., in the forensic framework, for pharmaco-toxicological analysis, or to deepen the knowledge on phytocannabinoids disposition and pharmacokinetics[3].

Straight-forward, yet efficient pretreatment protocols for whole blood microsamples obtained with capillary- and microfluidic-based technologies were developed, optimised, and coupled to an original LC-MS/MS method for the simultaneous analysis of THC, CBD and their metabolites, namely OH-Δ9-THC, Δ9-THC-COOH, 6α-OH-CBD, 7-OH-CBD, 7-COOH-CBD. The validation of these novel approaches provided good results in terms of extraction yield (> 84%) and precision (%RSD <6.9). To demonstrate the reliability of the developed microsampling methodologies, these were compared with a reference method for classic fluid plasma analysis. Then, microsampling coupled to LC-MS/MS was applied to real samples coming from users of non-psychoactive, CBD-based cannabis products. The obtained results proved the applicability of the developed microsampling platform for the accurate, straight-forward and reliable assessment of circulating THC and CBD together with their metabolites in several bioanalytical and pharmaco-toxicological frameworks.

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PF06

A multi-tissue mercury determination in a commonly consumed elasmobranch (*Mustelus mustelus*) of the Mediterranean Sea

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The concentrations of mercury (Hg) in the ocean have increased in the last decades through natural and anthropogenic sources. Hg is a highly toxic element that bioaccumulates and biomagnifies its concentrations mainly via diet in marine organisms[1]. In the marine environment, Hg is transformed into methylmercury (MeHg) by biological and chemical pathways. MeHg can comprise up to 90% of total Hg concentrations in fish muscle[2]. The smooth-hound shark (*Mustelus mustelus*) is commonly caught and consumed in Mediterranean countries. We aimed to determine Hg concentrations in the muscle, fin, and liver of *M. mustelus* around the Egadi Islands, Sicily (Fig 1). Hg concentrations were measured in different tissues by thermal decomposition amalgamation atomic absorption spectrometry. Our results showed that the highest median Hg concentrations were found in muscle 0.23 (0.09-1.20 mg/kg) and liver 0.10 (0.05-3.55 mg/kg), whereas the lowest in fin 0.01(0.004-0.102 mg/kg) (Fig 2). All individuals analysed were below the limits (1 mg/kg) allowed by the WHO and FAO, except for one specimen. Higher concentrations of Hg in the muscle may be due to its affinity for sulfhydryl groups associated with amino acids containing thiols[2]. Liver showed lower Hg concentrations than muscle, which could be explained by high selenium concentrations in shark livers; which has an antagonistic effect on Hg and contributes to detoxification[3]. Low Hg concentrations in the fin could be related to the low metabolic bioaccumulation rate of Hg in the composition of this tissue (cartilage/skin). A positive relationship was observed between Hg and size/biomass. In liver and fin, maturing individuals had higher Hg concentrations than juveniles. Immature individuals showed no differences in Hg concentrations between sex in any tissue, whereas, for adults due to the low number of individuals, it was not possible to determine. Nonetheless, the few male adults had higher Hg than females. Since *Mustelus spp*. are viviparous, this observation suggests a maternal-foetal transfer of Hg during gestation, reducing the concentrations in adult females relative to males. The analysed sharks do not represent a risk to human health; however, further studies are needed to fill the gap on bioaccumulation processes in adults.

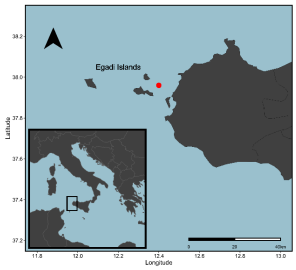

Fig. 1. Sampling location around the Egadi Islands, Sicily (Central Mediterranean Sea).

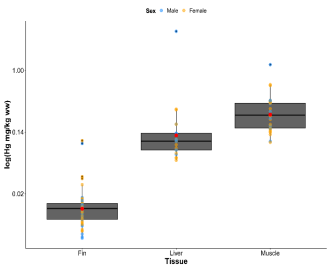

Fig 2. Boxplot o f fin, liver, and muscle Hg concentrations (y axis in log scale). The bold lines represent the median and red dots the mean.

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PF07

Untargeted characterization of phenolic compounds for varietal and geographical classification of industrial hemp

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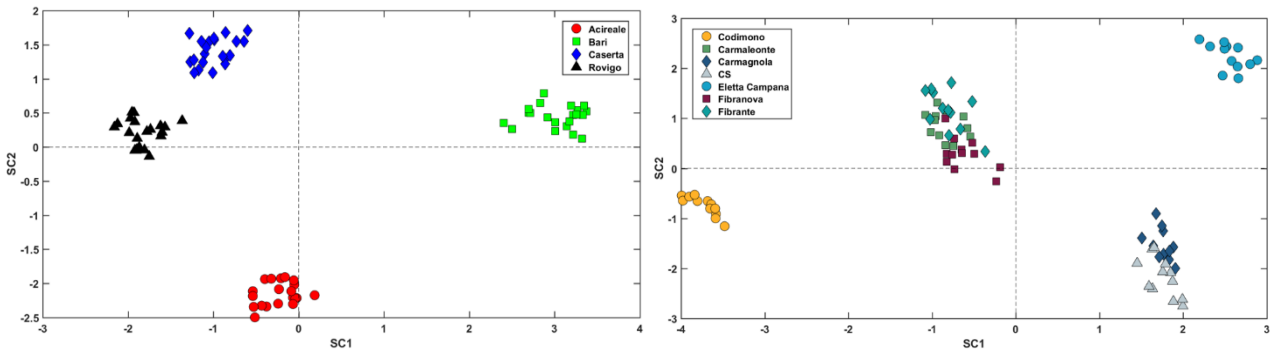
Industrial hemp (*Cannabis Sativa L.*) is an ancient, cultivated plant from Central Asia that has found several applications as it grows in variable habitats, soils, altitudes, and climate conditions [1]. Due to its secondary metabolism, hemp is characterized by a complex chemical composition, and it contains over 400 bioactive components such as cannabinoids, terpenoids, sugars, alkaloids, stilbenes, polyphenols, and quinones. Cannabinoids represent the main constituents of cannabis, and the most notorious compounds are Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) which have gained significant interest within the scientific community for their powerful biological activities [2]. Among the major constituents of cannabis, phenolic compounds have shown many beneficial effects on human health, such as main antioxidant and anti-inflammatory properties related to chronic diseases [3]. Recently cannabis research has extended its focus on other constituents, such as phenolic compounds, rather than the pharmacologically active cannabinoids. Indeed, common targeted approaches to a few major compounds might not be sufficient for characterizing cannabis varieties. At present, industrial hemp has been scarcely investigated compared to drug-type cannabis, despite its significant content in functional food ingredients and nutraceuticals and the progressive relaxation of the prohibition laws worldwide [4]. In the present work, an untargeted UHPLC-HRMS metabolomics workflow followed by suspect screening data processing was employed for assessing the role of phenolic compounds, particularly flavonoids, discriminating industrial hemp samples of different varieties and geographical origins. The method was developed on five dioecious (Carmagnola, CS, Eletta Campana, Fibranova, Fibrante) and two monoecious (Codimono, Carmaleonte) hemp varieties that were cultivated in 2021 in Rovigo, Bari, Caserta, and Acireale (Italy) with the application of no chemicals except nitrogen fertilization and water supply as needed. First, UHPLC-HRMS analysis was performed on all samples. Then, raw data were processed by Compound Discoverer 3.0; however, due to the structural inconsistency among flavonoids, the software was implemented with a comprehensive customized database generated ex novo resulting in a powerful tool for semi-automatic identification. In this work, a total of 132 flavonoids were tentatively identified, which represents the highest number detected in cannabis by an untargeted metabolomics-based approach present in literature so far. The use of chemometrics to interpret the chemical variability of samples suggested that crops tend to display different panels of secondary metabolites according to their varieties and pedoclimatic conditions.



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PF08

Chemical composition of European tea leaves: elemental content and health risk assessment for consumers

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Tea (*Camellia sinensis*) is the second most consumed beverage worldwide, playing a key role in the human diet. According to FAO, world tea production increased from 6.3 million tons in 2020 to an estimated 6.5 million tons in 2021, while the European annual consumption of tea ranges from 0.02 (Greece) to 3.16 (Turkey) kg *per* capita [1]. Tea is considered a healthy drink as its consumption is linked to a lower risk of cardiovascular disease–related events and death, stroke, metabolic syndrome, and obesity [2]. However, several studies have shown that *C. sinensis* is an hyperaccumulator of Al and other elements which are considered potentially toxic [3]. In the present study, the content of 15 elements (both essential and toxic) on 13 samples of tea leaves, collected in 6 different tea gardens (**Figure 1**), was measured for the first time in European teas through the atomic absorption spectroscopy technique. Results showed that the major element detected (g kg^{-1}) was Al (2±1), followed by Mn (0.3±0.2), Fe (0.08±0.02), Zn (0.029±0.007), Cu (0.016±0.003), and Ni (0.010±0.005). Other elements detected with a lower concentration (mg kg^{-1}), were Cr (0.8±0.5), Pb (0.6±0.2), V (0.1±0.4), Co (0.1±0.2), Se (0.11±0.02), As (0.098±0.007), Cd (0.03±0.02), Ag (0.007±0.001), and Hg (0.006±0.004). Some elements showed a statistically significant correlation ($p<0.05$) in their distribution: the pairs Al–Mn, Cr–Zn, Hg–Mn, Pb–V (positively correlated) and the pairs Al–Fe, As–Ni, Cu–Hg (negatively correlated), highlighting mechanisms of synergic or antagonist interaction. Moreover, the principal component analysis (PCA) revealed that the geographical origin was the main driver clustering the samples (**Figure 2**), while the treatment process (black and green) did not significantly affect the content of elements in the leaf. This is probably due to a site–specific effect connected to the different environmental conditions that influence the plant during the growth, such as the soil chemistry used for cultivation. Further studies are planned to clearly establish the weight of this aspect on the pathway of distribution of these elements. Moreover, the health risk assessment was evaluated using the Hazard Quotient (HQ) and the Hazard Index (HI), highlighting no risk on the consumption of these products by Europeans.

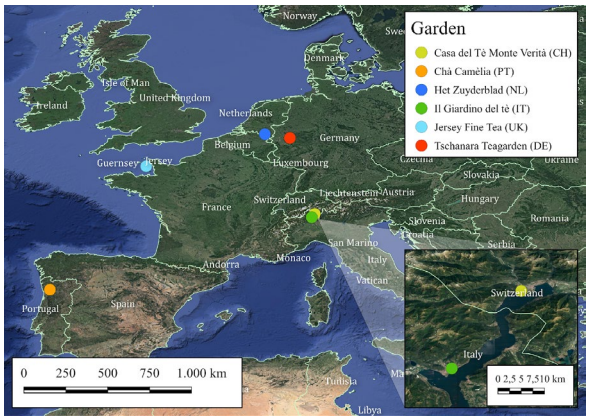

Figure 1. European tea garden location.

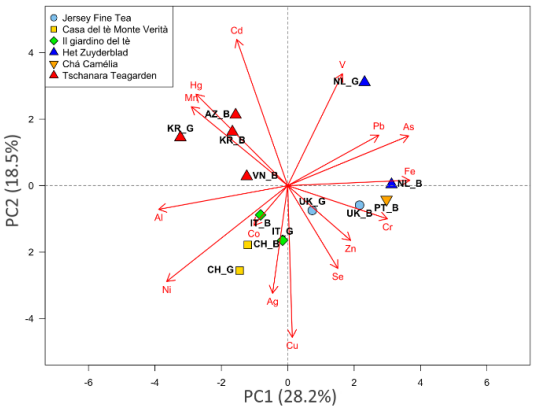

Figure 2. 2D biplot of PC1 *vs* PC2.

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PF09

Electrochemical cell-free biosensors for antibody detection

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Antibody detection is important in several clinical settings because it informs on current and past infection and can provides information about clinical outcomes. Synthetic biology has been proposed as a way to expand medical monitoring and diagnostics of human diseases. In this prospective, cell-free devices would bring new capabilities to diagnostics methods by creating sensors with new functions, expanding the range of targets, and improving sensitivity and specificity. In recent years, cell-free biosensors for the detection of nucleic acid, small molecules, and proteins, including antibodies,[1-4] have been developed with excellent sensitivities and specificities. While these examples present several advantages, they remain bound to the limitations typical of optical methods: low performance in complex sample matrices and lack of portable and low-cost instrumentation. Here we propose an electrochemical cell-free biosensor for antibody detection directly in complex sample matrices particularly suitable for point-of-care diagnostics. [5] The approach is based on the use of programmable antigen-conjugated gene circuits that, upon recognition of a target antibody, trigger the cell-free transcription of an RNA sequence that can be consequently detected using a redox-modified strand immobilized to a disposable electrode (Figure 1). The system we proposed couples the advantageous features of cell-free diagnostic methods (i.e., sensitivity) with those of electrochemical platforms (i.e., portable, inexpensive, disposable sensors).

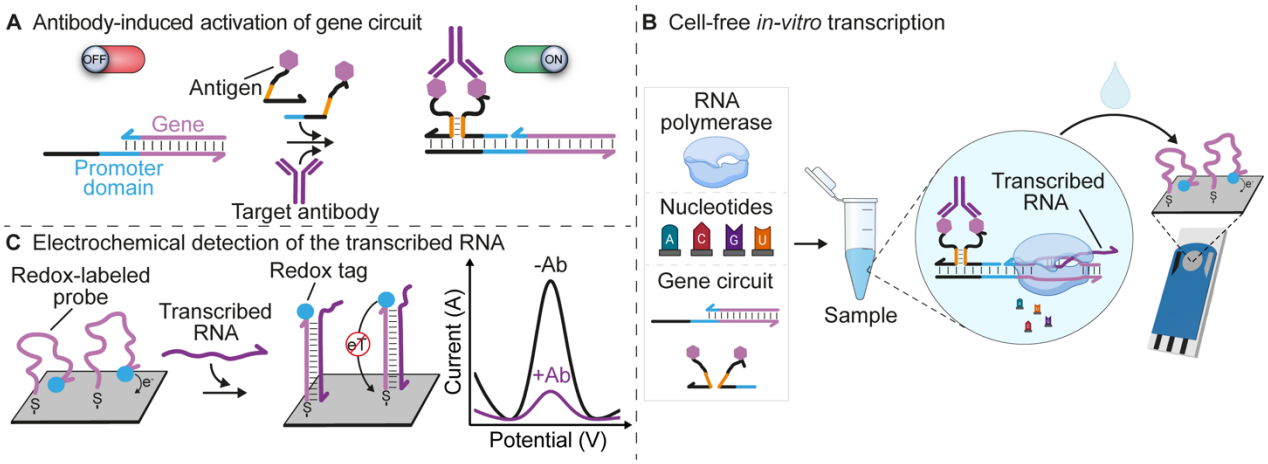

Figure 1. Electrochemical cell-free biosensor for antibodies detection.

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PF10

Analysis of Trace Elements: Eurasian Otter (Lutra lutra) an Expanding Sentinel Species in Friuli Venezia Giulia Region

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Anthropogenic activities over the years have caused a progressive release into the environment of heavy metals and metalloids whose persistence and potential for bioaccumulation have adverse effects on humans and wildlife. The latter has been shown to be sensitive to the presence of pollutants and to react by changing its distribution and presence. The Eurasian otter (*Lutra lutra* L., 1758) is a medium-sized piscivorous animal with high dietary plasticity, making it one of the top predators in aquatic and riparian ecosystems. The European population collapsed due to anthropogenic threats and -A_strong correlation with the evidence of pollutants has been reported (Foster-Tuley et al., 1990). Due to its sensitivity to pollutants, ecological role as an apex predator, longevity, site fidelity, the otter can be used as a sentinel species to measure the quality and state of health of the ecosystems in which inhabits (Wainstein et al., 2022). The study area is the Friuli Venezia Giulia Region (Figure 1), which was recently recolonized by *L. lutra* after disappearing for more than 40 years. The study focuses on the analysis of trace elements in spraints samples (i.e., otter scats) collected in three areas: downstream the mining sites of Raibl on the Slizza basin and of Idrija on the Isonzo basin, and in a reference one on the Fella-Tagliamento basin. Analyses were carried out on 29 spraints collected on 7 rivers representative of the study area (in Figure 1: torrente Slizza - *tS*, rio Vaisonz - *rV*, fiume Fella - *fF*, torrente Resia - *tR*, canale Bonifica – *cB*, fiume Ledra - *fL*, fiume Vipacco - *fV*). Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) and Direct Mercury Analyzer (DMA–1) were applied to measure 27 trace elements and total mercury content, respectively. Subsequently, data were elaborated using R and Quantum GIS software to obtain pollutants' distribution in the study area, and finally compared with literature data (Baos et al. 2022, Rodríguez-Estival 2020) even if from different areas. This approach of environment monitoring is for the first time applied in our area and the results represent a baseline even if, some of the areas are potentially highly contaminated for mining activities. The analyses confirmed the presence of trace elements in the study area (Tab. 1), mainly As, Cd, Hg, Pb, and Zn. Just to offer some order of magnitudes, the limit for Hg as by EPA (Jen 2023) is 2 ppb in drinking water and the WHO recommended safe limits of Hg in wastewater and soils for agriculture are 0.0019 and 0.05 ppm respectively, as for Pb the limit in drinking water is 0 µg/L and in wastewater is 0.05 ppm; as for foodstuff the legal limits of Pb and Hg are 1,5 and 200 mg/kg wet weight respectively((EC) No 1881/2006). Particularly, a higher average concentration of As and Hg is measured in samples from the Vipacco river area, adjacent to the historic Mercury mines of Idrjia. For Cd and, in particular, Pb and Zn are most prevalent in the Slizza stream, a river in which, along its banks there are still deposits of waste from the mines of Raibl. Contamination of environmental matrices travels up the trophic chain of the aquatic ecosystem, eventually accumulating in fish, the elective prey of otters, which can accumulate trace elements. The results show that otters have repopulated an area where pollution is still detectable. However, it is believed that due to the stop of the activities, pollution decayed over years justifying the re-appropriation of the area by the otters. Pollutant levels in Eurasian otter scats are reflective of regional abiotic and biotic conditions, therefore otters represent an excellent biomonitor of food web and environmental contaminant exposure in the river system (Wainstein et al., 2022) using them as a sentinel species of the quality of the Ecosystem.

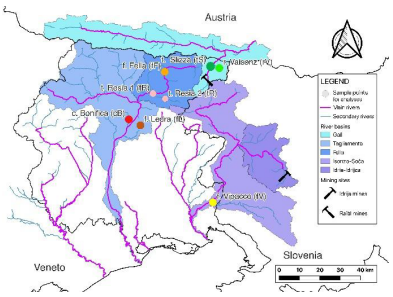

Table 1. Concentrations of As, Cd, Hg, Pb, and Zn in the most contaminated rivers, compared to previous studies (median and range, reported in μg g^{-1} d.w.).

Study areas / Trace elements (μg g^{-1})	As	Cd	Hg	Pb	Zn	Reference
Torrente Slizza, Italy	2.69 (<LOD-8.83)	0.77 (0.07-1.14)	0.12 (0.09-0.18)	92.14 (32.12-259.85)	1353.44 (656.74-1749.04)	This study
Rio Vaisonz, Italy	1.92 (<LOD-6.23)	1.79 (<LOD-6.39)	0.16 (0.13-0.20)	6.76 (<LOD-27.02)	676.83 (622.03-777.00)	This study
Fiume Vipacco, Italy	2.38 (1.85-3.19)	0.41 (0.25-0.67)	0.89 (0.78-1.00)	2.97 (0.38-8.42)	547.36 (307.22-727.35)	This study
Torrente Resia (Ref Area), Italy	0.39 (<LOD-0.79)	0.26 (0.11-0.63)	0.14 (0.05-0.24)	0.19 (<LOD-0.38)	426.34 (340.29-573.84)	This study
Valdeazogues (Hg Area), Spain	NA	NA	1.88 (0.80-8.17)	0.15 (<LOD-13.87)	NA	Rodríguez-Estival et al., 2020
Montoro river (Pb Area), Spain	NA	NA	0.23 (0.10-0.34)	6.55 (0.55-282.90)	NA	Rodríguez-Estival et al., 2020
Bullaque river (Ref Area), Spain	NA	NA	0.15 (<LOD-0.35)	0.93 (0.24-4.06)	NA	Rodríguez-Estival et al., 2020
Guadiamar river (Contaminated Area), Spain	0.29 (0.01-4.19)	4.20 (2.50-7.50)	NA	8.60 (4.51-18.50)	587.70 (248.50-1276.00)	Baos et al., 2022
Guadalete river (Ref Area), Spain	NA	2.40 (1.60-4.20)	NA	0.40 (0.02-1.30)	330.10 (137.30-901.20)	Baos et al., 2022

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PF11

Paper card-like electrochemical platform for point-of-care detection of glucose in tears

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The growing demand for user-friendly and affordable analytical tools in the healthcare sector is driving the development of reliable platforms for the customization of therapy depending on individual health conditions. Paper-based point-of-care (POC) tests are now one of the most efficacious and accessible tools for real-time monitoring of diseases. In this regard, we have developed a paper card-like bioplatform for the on-site monitoring of glucose in tears. This noninvasive approach relies on the employment of a disposable cellulose paper that serves as reservoir for glucose oxidase (GOx) and electrolytes loading, inserted into a home-made polyvinyl chloride (PVC) electrochemical system with three connected electrodes. To achieve better sensitivity, the hydrophilic area on the paper card was modified with a dispersion of carbon black and Prussian blue nanoparticles. The main feature of the developed device is the fact that by inserting a pre-loaded paper card for each measurement, the PVC-based electrochemical cell can be reused for several measurements without the need to renew the electrodes. Under the optimized conditions of nanoparticles volume (20 μL) and enzyme concentration (1.2 U), the developed amperometric device enables the detection of glucose in the artificial tears in the range of 0.2-2 mM with a detection limit of 50 μM (RSD=6%). In addition, we assessed the usefulness of the developed device to detect 0.5 mM glucose in the presence of lysozyme whose concentration differs widely from basal to reflex tears. The calculated recoveries were equal to 92% ± 8 and 94% ± 5, respectively, for simulated basal and reflex tears, demonstrating the absence of the lysozyme effect. Moreover, a variation within the experimental error was obtained when testing different paper batches, which proved the robustness of the paper card-like device that was stable for at least 7 days at room temperature. To enlarge the application of the developed device, we are considering the analysis of human's tears collected from people with different health conditions.

Keywords: Paper card-like; Polyvinyl chloride; Glucose; Tears; Glucose oxidase; Carbon black; Prussian blue nanoparticles; Electrochemical sensing; Portable device.

PF12

Size separation and characterization of human polysomes by flow field-flow fractionation

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A polyribosome (or polysome) is a structure of chained ribosomes bound to an mRNA molecule. It consists of an mRNA molecule and two or more ribosomes that act to translate mRNA instructions into polypeptides. Isolated polysomes (also known as translating ribosomes or polyribosomes) are mRNA–ribosome complexes that are frequently used for the in vitro study of the regulation of protein synthesis.

Polysome profile analysis is a popular method for separating polysomes and ribosomal subunits and is typically achieved by sedimentation on a sucrose density gradient (SDG) followed by gradient fractioning coupled to a UV detector. This has remained the gold standard method since ribosomes were first discovered; however, this method is time-consuming and requires multiple steps from making the gradient and long ultracentrifugation to analyzing and collecting the fractions [1].

Asymmetric flow field-flow fractionation (AF4) is a subtechnique of field-flow fractionation that separates molecules according to differences in their diffusion coefficient, which reflects their size and shape. In combination with on-line detectors like UV/vis, fluorescence and multi-angle light scattering, AF4 provides an analytical platform able to separate and characterize biological macromolecules and particles. AF4 has been applied to a wide range of biological analytes [2], including plant and bacterial ribosomes [3].

In this study, we present an AF4-multidetection method for the separation and characterization of human ribosomes and polysomes. The running conditions are set to obtain, in a single run and short analysis time, size resolution of ribosomal subunits and polysomes.

Size-uniform polysome fractions are collected for further, offline characterization. This can consist in low or high throughput analysis of either mRNAs recruited to polysomes or of the proteins constituting or binding ribosomes (ribosomal proteins, translation factors, regulatory proteins, etc.). This kind of analysis allows to define the events which regulate gene expression at the post-transcriptional (translational) level.

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PF13

A paper origami platform as a sustainable analytical approach for multiplexed analysis in urine

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Sustainability is an ongoing emergency that claims the attention of all scientific sectors. To get closer to the principles of sustainability, the modern views in Analytical Chemistry (i.e., Green Analytical Chemistry [1] and White Analytical Chemistry [2]) have recently defined the criteria for developing sustainable analytical methods, including the realization of devices suitable for multiplexed analysis, composed of eco-friendly materials, and characterized by affordable costs, easiness of usage, and simplicity of procedures. A significant breakthrough in this direction has been represented by the introduction of paper as a key component in sensor development.
Herein, we have combined different properties of the paper to simultaneously address the sustainability principles, thanks to an origami-like paper platform for multiplexed analysis in biofluids (i.e., human urine) in health care monitoring. Considering that uric acid is a key biomarker for kidney diseases that can be associated with health risks due to pesticide exposure, the sensing principle was specifically designed for the dual detection of uric acid and glyphosate pesticide. In detail, a paper strip was used as a porous substrate, designed to drive the sample through a lateral flow configuration. The paper's porosity was exploited to pre-load all the needed reagents and treat the sample within the cellulose itself. After the treatment of the urine sample, uric acid was detected by direct oxidation in differential pulse voltammetry while glyphosate detection was accomplished by an enzyme inhibition assay, using horseradish peroxidase enzyme and TMB as the substrate. As a key point, the additive contribution of uric acid and glyphosate to horseradish peroxidase inhibition was studied and addressed to enable their detection. To carry out the electrochemical measurements, office paper was used as a low-cost and green substrate for graphite-based screen-printed electrodes, modified with a carbon black dispersion. Two office paper sensors were assembled with the paper strip, allowing for the sequential detection of uric acid and glyphosate with a linear range of 0.1 - 1.5 mM and of 100 - 700 ppb, respectively. The sustainability of our sensing platform was assessed by considering the principles of White Analytical Chemistry [2], in comparison to reference methods for uric acid and glyphosate detection in urine, highlighting the advantageous aspects brought by our approach.
Overall, the here presented sensing platform is an emblematic example of how the paper can be exploited for developing innovative analytical solutions suitable for multiplexed analysis in biofluids, which meet the requirements of sustainable analytical methods.

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PF14

Design of a printed electrochemical strip towards miRNA-21 detection in urine samples: optimization of the experimental procedures for real sample application

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MiRNAs are noncoding RNAs around 22 nucleotides in average length. MiRNAs play an essential role in biological activities, including cell proliferation, development, differentiation, metabolism, apoptosis, signal transmission, organ development, hematopoietic lineage differentiation, host-viral interactions, and tumorigenesis [1]. Research shows that miRNAs play an important role in the development of various diseases, including oncogenesis and metastasis [2–3]. The presence of specific miRNA in various bodily fluids makes them circulating biomarkers of high interest for the research in the field of liquid biopsy [4]. In this work, an electrochemical detection platform for miRNA based on a screen-printed gold electrode was developed. A DNA sequence modified with methylene blue (MB) was covalently bound to the electrochemical strip [5]and used to detect the selected target miRNA-21. After optimization of selected parameters in standard solutions, including the study of the effect of pH, presence of interferent species and NaCl salt concentration in the background, the application of square-wave voltammetry (SWV) technique allowed the detection of miRNA-21 down to a limit in the order of 10 nM. The developed device was then applied to several urine samples. In this case too, the device showed high selectivity in the presence of the complex matrix, satisfactory repeatability and a limit of detection in the order of magnitude of 10 nM, similarly as what observed in standard solutions.

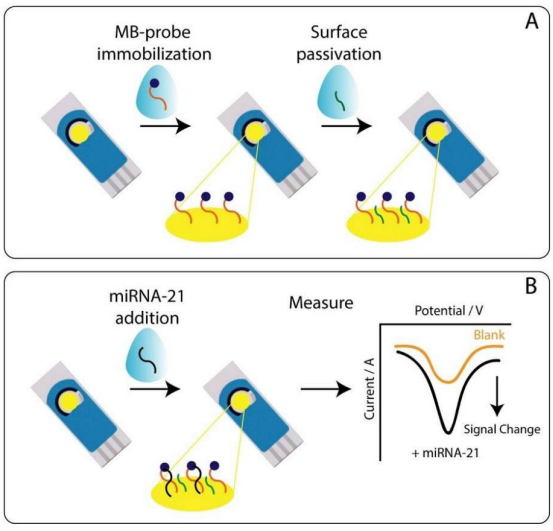

Figure 1: A) Schematic representation of electrochemical device fabrication and B) principle of miRNA-21 detection.

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PF15

An *Origami* Paper-Based Biosensor for Allergen Detection by Chemiluminescence Immunoassay on Magnetic Microbeads

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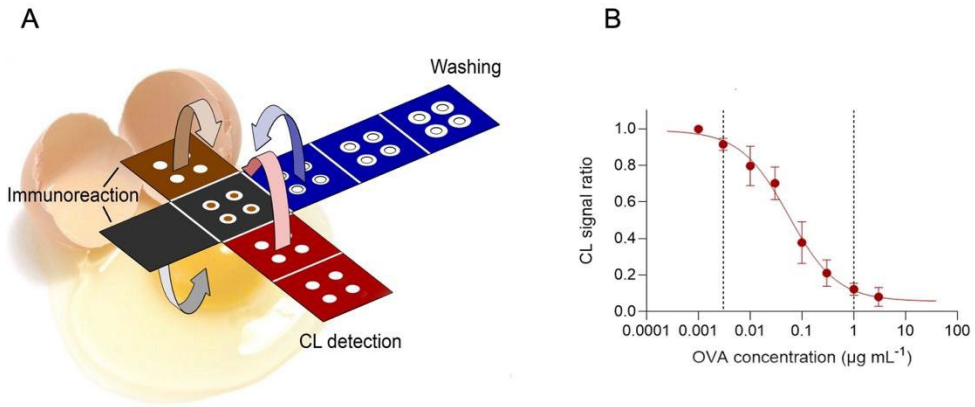
Food allergies are adverse health effects that arise from specific immune responses, occurring upon exposure to given foods, even if present in traces [1]. Egg allergy is one of the most common food allergies, caused mainly by egg white proteins, of which ovalbumin being the most abundant [2]. There are several analytical techniques for determining allergens in food, the most widely used being immunometric (i.e., ELISA), molecular (i.e., RT-PCR), and instrumental methods (i.e., LC-MS/MS) [3]. All of them, however, rely on expensive laboratory techniques, involve long and complex protocols, and require specialized personnel. Since cross-contaminations inevitably occur in the food production chain, cheap, rapid, and field-deployable biosensors are required for monitoring the presence of allergens. Herein, we report an *origami* paper-based device for detecting ovalbumin in food samples, based on a competitive immunoassay with chemiluminescence detection using a portable CCD-based detector [4]. In this biosensor, magnetic microbeads have been employed to easily and efficiently immobilize ovalbumin on paper. As a result, immobilized ovalbumin competes with the ovalbumin present in the sample for a limited amount of enzyme-labelled anti-ovalbumin antibody. By exploiting the *origami* approach, a multistep analytical procedure could be performed using reagents preloaded on paper layers, thus providing a ready-to-use immunosensing platform (Fig 1A). The advantage of this format is to exploit the separation and purification of the analyte from the sample matrix on magnetic microbeads, which can be easily recovered from their suspensions by applying a magnetic field. This soft sample isolation approach simplifies and speeds up the incubation and washing steps of ELISA tests, thus facilitating the transfer of such methods into miniaturized formats, such as in paper-based or microfluidic devices. The assay provided a limit of detection (LOD) of about 1 ng mL^{-1} for ovalbumin (Fig 1B) and, when tested on ovalbumin-spiked food matrices (chocolate chip cookies), demonstrated good assay specificity and accuracy, as compared with a commercial immunoassay kit.



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PF16

A 3D-printed electrochemiluminescent enzymatic glucose biosensor based on luminol-H_2O_2 system and smartphone detection

Donato Calabria[a], Elisa Lazzarini[a], Andrea Pace[a], Ilaria Trozzi[a], Martina Zangheri[a], Stefano Cinti[b], Marinella Difonzo[a], Giovanni Valenti[a], Massimo Guardigli[a], Francesco Paolucci[a], Mara Mirasoli[a]

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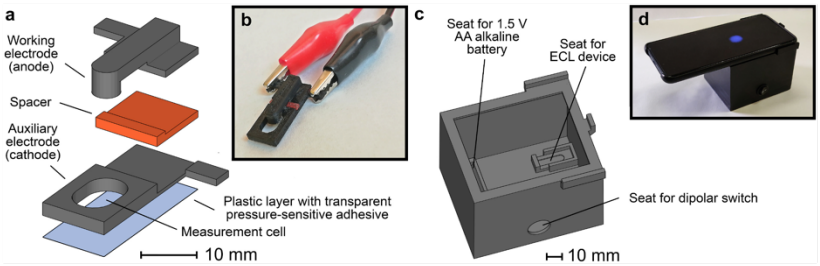
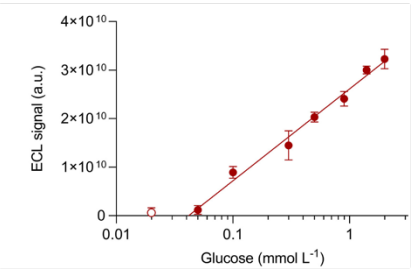
Electrochemiluminescence (ECL) is a luminescent phenomenon in which photons are emitted upon the decay of an excited state species, arising from a highly energetic electron transfer reaction between electrogenerated radicals [1]. In recent years there is a growing interest in the development of Point-of-Care Testing (POCT) biosensors based on ECL, owing to its several advantages such as reproducibility, high sensitivity, selectivity, and broad linear range with lower detection limit [2]. Three-dimensional (3D) printing has attracted immense attention in the development of biosensors, because it enables rapid prototyping, waste minimization, design customization, and low-cost manufacturing [3]. We present the development of an ECL biosensor based on a 3D printed electrochemical cell for the detection H_2O_2, exploiting the luminol/ H_2O_2 luminescent system. As H_2O_2 is the by-product of oxidases, specific enzymes that catalyze oxidation-reduction reactions of several biomarkers, various bioanalytical applications can be envisaged. The electrochemical cell is obtained by Fused Deposition Modelling (FDM) 3D printing, employing conductive black polylactic acid (PLA) for producing the electrodes. The ECL signal was imaged and quantified employing a CMOS smartphone camera and a simple 1.5V battery was used as power supply of the electrochemical system (Fig 1A). As proof-of-concept, we developed an ECL biosensor to quantify glucose in pharmaceutical preparations and biological samples. Under the optimal conditions, a detection limit of 8.0×10^{-6} mol L^{-1} and 1.0×10^{-5} mol L^{-1} was obtained for H_2O_2 and for glucose, respectively. The linear response range of glucose was 5.0×10^{-5} to 2.0×10^{-3} mol L^{-1} (Fig 1B). The ECL sensor showed a good reproducibility and long-term stability. The proposed ECL biosensor could be applied to other analytes exploiting several oxidases, it could be improved due to the simplicity to change the form of electrodes by 3D printing, and it is suitable for the miniaturization because of the simplicity of the equipment required for ECL trigger and detection.

A



B



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Presentazioni Poster

P01

Bioluminescence analytical method based on 3D microtissues for the evaluation of different bioactivities of water samples

Maria Maddalena Calabretta[a,b], Denise Gregucci [a,b], Marina Bonini[c], Elisa Neri[c], Martina Zangheri[a], Elisa Michelini [a,b,d]

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The development of predictive *in vitro* sensing tools able to provide rapid information on the different bioactivities of a sample is of pivotal importance, not only to monitor environmental toxicants, but also to understand their mechanisms of action on diverse molecular pathways. This mechanistic understanding is highly important for the characterization of toxicological hazards, and for the risk assessment of chemicals and environmental samples such as surface waters and effluents. Prompted by this need, we developed and optimized a straightforward bioluminescent multiplexed assay which enables the measurement of four bioactivities, selected for their relevance from a toxicological perspective, in bioluminescent microtissues.[1] The assay was developed to monitor inflammatory, antioxidant, and toxic activity, and the presence of heavy metals, and was successfully applied to the analysis of river water samples, showing potential applicability for environmental analyses. The assay, which does not require advanced equipment, can be easily implemented in general laboratories equipped with basic cell culture facilities and a luminometer.

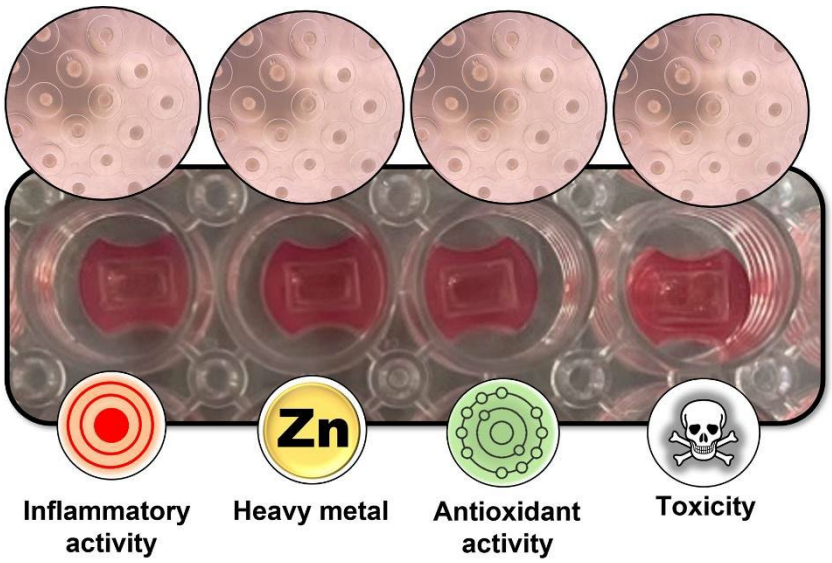

Figure 1: Schematic representation of the bioluminescence sensing platform relying on 3D spherical microtissues for multiple bioactivity analysis

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P02

A comprehensive evaluation scheme and quality control and purification of ready-to-use conjugated gold nanoparticles based on Asymmetric Flow Field-Flow Fractionation (AF4)

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The study of the interaction between gold nanoparticles (AuNPs) and proteins helps to better understand their intrinsic behaviors, which provides valuable information for the development of systems interesting in the field of imaging, drug delivery, and photodynamic and photothermal therapy. Current literature lacks procedures able to separate, purify and characterize these species in native conditions without altering them while assuring a high throughput. In this work, an analytical method based on asymmetric flow field flow fractionation (AF4) with photodiode array and multiangle light scattering detector (AF4-DAD-MALS) has been exploited to study the binding behavior of proteins (BSA) on the surface of AuNPs protected by different types of surface coating (PEG and citrate).

The AF4 platform provided a full separation of particle-protein conjugates at different compositions, pH, and ionic strength to clarify their effect on conjugate's stability and binding equilibrium. MALS results combined with the information obtained from batch dynamic light scattering (DLS) measurements and FFF theory calculations allowed a complete molecular weight, size, and stoichiometry-wise characterization of the samples. At the same time, a robust but flexible method can be extended to other kinds of proteins and ligands ranging from 10 to 200 kDa, and for the development system of interest.

Overall, the analytical strategy and experimental scheme in this work provide a general and comprehensive set of evaluation criteria for metal-protein conjugates and an important feature for the quality control required to optimize bio-probe synthesis and derived bioassays.

P03

Natural neurotransmitters as “green” functional monomers for protein imprinting

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Molecular imprinting and related technologies are becoming increasingly appreciated in bioanalysis and diagnostic applications. MIPs (Molecularly Imprinting Polymers) are defined as synthetic receptors for a targeted molecule and are an interesting alternative perspective to classical bioreagents (i.e., antibodies), which are often very expansive, sensitive to environmental conditions and need to use laboratory animals for their production. On the contrary, the imprinted polymers are synthesized by a standardized *in vitro* process, not involving organism, and potentially offer the specificity and selectivity of the biological receptors with the explicit advantages of durability with respect to environmental conditions, low cost, and reduction of batch-to-batch variability [1,2]. Among the imprinted polymers, we have been exploring endogenous neurotransmitters as nature and sustainable building blocks (monomers) to straightforwardly design and synthesize highly selective and sensitive imprinted biopolymers (IBPs) of the new generation. Inspired by nature, they derive directly from the polymerization of the endogenous neurotransmitters (NTs) dopamine (DA) and norepinephrine (NE) and, very recently, serotonin (SE). These self-polymerizing functional monomers have the advantage of circumventing the use of cross-linkers, usually associated with potential toxicity, thus limiting clinical applications. Moreover, they exhibit important adhesive properties both to organic and inorganic surfaces and its production is one step and fast.

The strategy used for these IBPs is the so-called epitope imprinting approach, it is based on the use of a short “fingerprint” synthetic peptide (few amino acids 10-15 are rationally selected), as a template, that constitutes only a small portion of the large antibodies to which belongs, and that, in turn, may be recognized by the imprinted polymer. This technique allows achieving unexpected advancements (and reagents saving) in the recognition of large biomolecules, by mimicking the efficacy of the natural binding of antibodies.

In this presentation we will summarize the last important achievements by developing these innovative bio-inspired materials, with a focus on the newest on, polyserotonin (PSE). Its potential ability in forming the related polymer (polyserotonin, PSE) is still virtually absent in the literature, excluding very few recent works [3, 4]. No one, to the best of our knowledge, has attempted the molecular imprinting of PSE. In this scenario, we investigated for the first time the ability of SE in forming adhesive non-imprinted and imprinted nanofilms for bioanalytical purposes and compared its imprinting efficiency with those of PNE and PDA, under the same imprinting and binding conditions. As a model study, tumor necrosis factor-alpha (TNF-α) was selected as a biomolecular target of interest in clinical diagnostics. The biomimetic receptors were coupled to an optical real-time and label-free Surface Plasmon Resonance (SPR) and the quantifications were performed both in buffer and biological matrices.

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P04

Monoclonal antibodies (mAbs) optical detection by coupling innovative imprinted biopolymers and magnetic beads: the case of therapeutic mAb anti-myostatin detection

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Immunotherapy has revolutionized modern medicine, becoming the largest part of the growing bio-drugs market. Currently, the available monoclonal antibodies (mAbs) have been applied to several therapeutic fields, including autoimmune disease, asthma, infection, and cancer [1]. Along with the progress in this direction, the need for reliable mAbs monitoring methods is a current foremost issue. In fact, from a point of view of analytical methods, at the present time, only classical immunochemical assays are available for the determination of immunotherapics, based on expensive and fragile capture antibodies. Accordingly, the goal of this study was to design a straightforward beads-based plasmonic approach that combines magnetic beads (MBs) with a polynorepinephrine imprinted biopolymer (IBP) for real-time mAbs detection in biological matrices. Specifically, MBs-encoded by a specific antigen were exploited not only for the selective capture of the target mAb from human serum, but also MBs are directly involved in the molecular architecture of a sandwich assay. The mAb extraction from the real matrix occurred in a site-oriented manner by exploiting a paratope-epitope specific recognition, while leaving the constant mAb fragment (Fc) free to interact with an IBP specific for the mAb Fc portion.

In this scenario, MIPs are proving to be very effective both in terms of technical performance and versatility, cost, and stability [2]. In details, we have been chiefly focusing on norepinephrine (NE) as functional monomer which is becoming increasingly appreciated as biocompatible material in accordance with its considerable advantage. In fact, polynorepinephrine (PNE) compared to polydopamine (PDA) shows lower hydrophobicity of surface, which minimizes unspecific binding and allows a fully reversible regeneration of the MIP surface [3]. The production of biomimetic receptor is relatively simple (by direct drop casting of the polymerizing NE solution on the SPR gold chip), cost-effective, fast (5h of polymerization) and does not require animal immunization.

As a case study, Stamulumab (MYO-029), a recombinant monoclonal antibody was addressed as biological target to evaluate the analytical performances of the beads-based sensing assay. MYO-029 is a monoclonal recombinant human immunoglobulin G (IgG1) developed for the immunotherapeutic treatment of muscular-wasting disorders, such as cachexia, sarcopenia, and muscle dystrophy. In detail, MYO-029 binds with a high affinity to myostatin and inhibits its activity [4]. Myostatin-neutralizing antibodies may be misused as performance-enhancing drugs in sports competitions and their effective quantification has a central role also in the anti-doping control field.The optimization of the assay conditions led to establishing an assay able to achieve very good analytical performances in terms of repeatability and sensitivity, with negligible cross-reactivity with other Ig classes and IgG subclasses. In this frame, we proposed an extremely modulable assay, with potential large applicability, for multiplexing mAbs detection.

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Acknowledgments

We wish to thank the Italian Ministry of Health for the financial support within the call "2020 Research and training/information program on drugs, medical substances and practices that can be used for doping purposes and for health protection in sporting activities" (project "Immunotherapy abuse in sport: development of a biosensor for myostatin inhibitors").

P05

Synthesis of nanoparticle-decorated biodegradable membranes for use in electro-membrane extraction techniques

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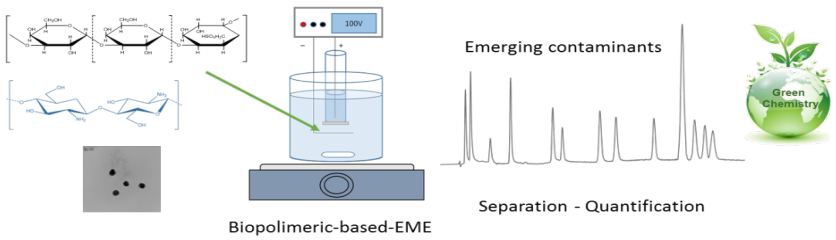
The development of more environmentally friendly analytical methodologies is currently a common goal when implementing new procedures. In this regard, microextraction procedures such as electromembrane extraction (EME), based on the use of alternative materials, have been proposed. In particular, EME is one of the most widely used sample preparation techniques, with diverse applications in the analysis of biological, environmental and food samples. It is characterised by facilitating the transport of matter by electrokinetic migration, whereby analytes possessing ionisable functional groups in their structure are extracted directly in their charged form (acidic or basic) through a supported (usually organic) liquid membrane (SLM) into an aqueous phase by the application of an external electric field [1]. With this technique, several groups of analytes (acidic and basic compounds, polar and non-polar, ions or drugs) have been determined in a multitude of formats and extraction devices (such as hollow fibres, flat membranes or microfluidic devices) using different types of supporting materials. Typically, these supports have been plastic materials such as polypropylene, both in the form of hollow fibres and flat membranes. Polyvinylidene difluoride or polyacrylonitrile have also been used, although to a lesser extent [2]. Within the field of green analytical chemistry, the use of alternative materials to classical plastics is a key objective for the synthesis of films or support membranes in EME techniques. In particular, biopolymer derivatives such as chitosan and agarose have recently experienced great importance as biodegradable and sustainable materials, which is a great advantage given the reduction of the environmental impact derived from these processes [3].

The new biopolymeric films synthesised will make it possible to replace the plastic supports traditionally used in EME processes with these new membranes which are biodegradable and sustainable due to their natural source, thus contributing to the trend towards a greener environment. Moreover, the functionalisation of these biofilms with metallic gold nanoparticles will allow the design of customized membranes, thus selecting the analytes to be extracted from complex matrices. Specifically, drugs belonging to different families (non-steroidal anti-inflammatory drugs (NSAIDs) or antibiotics), as well as other substances considered as emerging pollutants (parabens, among others) could be targeted extracted trough their interaction with the biomembrane.

Agarose-chitosan based membranes, decorated with metal nanoparticles, have been synthetized and succesfully applied in EME procedures for the selective extraction of NSAIDs, parabens and fluoroquinolones from biological and environmental samples. The presence of nanoparticles in the biomembrane improves the efficiency of the extraction (higher enrichment factors), enabling additionally a targeted and green methodology for the determination of different drugs.



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P06

Design for a platform for analysis and selective quantification of the amount of urea in wastewater and fluids of biological origin

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One of the primary byproducts of protein catabolism in humans is urea. Excessive urea levels in the blood can result in renal problems, gout, and other illnesses. Urea is excreted in urine by both humans and animals (428.4 to 714 mmol/day in the case of humans). Moreover, urea synthesis in industry and usage as a nitrogen-release fertilizer in agriculture can result in eutrophication events [1] and the creation of urea-containing wastewater. In the last few years, it has been possible to appreciate an increase in publications concerning the problems with this compound and how its concentration can be lowered in waters with excessive concentrations. The amount of urea in wastewater treatment facilities is reduced using a variety of methods. Examples include electrochemical oxidation using Ni, NiO, Ni/C, and NiCo/C-based electrodes, biotreatment using enzymes or bacteria, or adsorption on zeolites or nanostructured materials. As the last technique, hydrolysis using cascade coupled hydrolysis/desorption processes is also an option [2]. The primary aim in a wastewater treatment plant should be to recover components like urea or phosphate in order to valorise this waste. Urea is now generated from synthetic ammonium, which has a costly and negative impact on the environment. Using enzymes, nanozymes, and other catalysts, this PhD thesis aims to establish an electroanalytical platform for the in-stream determination of urea in sewage water. There are several instances when CO_2-permeable gas membranes, ISE for ammonium, and ion-selective electrodes for ammonia are used. There are also recorded instances of nanostructured amperometric sensors [3]. Recently, the use of this organic molecule has become increasingly popular in the study of possible applications for the development of fuel cells for hydrogen production. The technologies currently in use exploit as catalysts the same metals, alloys or mixtures that are used for quantification in liquid samples. The growing interest in the use of this possible resource, however, invites us to remain alert to the possible adverse effects it may have on the environment and thus to reveal the need for the development of new technologies and materials [4]. In this work, amperometric and potentiometric transducers are characterized with the aid of suitable cutting-edge "green" materials for the development of a urea sensor for on-line wastewater monitoring.

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P07

Development of an antigen lateral flow immunoassay for the on-field detection of African Swine Fever virus in target tissues

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African swine fever (ASF) is a severe haemorrhagic infectious disease caused by a large and complex double-stranded DNA virus (genus Asfivirus, family Asfarviridae). It affects suids, and is vehiculated by soft ticks, and transmitted to pigs and wild boars [1]. ASFV is not dangerous for humans but, can be carried making them act as reservoirs. The recent outbreak from Eastern to Central and Southern Europe has become a very high animal health and economic concern causing massive losses in terms money from pork products due the high contagiousness. The massive transboundary movements of boars promote the spread of the infection. The diagnosis of ASFV includes laboratory virus (based on polymerase chain reaction) and antibody (based on immunoassays) detection methods to confirm the infection. Nevertheless, early detection is crucial for such a contagious infectious disease, then rapid point of care tests (POCT) is required being powerful tools to control the spread of the disease as confirmed by their role in the recent COVID19 pandemic [2]. Many POCTs, based on molecular [3] (Recombinase Polymerase Amplification, rtPCR, LAMP) and immunochemical [4] (fluorescence and colorimetric Lateral Flow ImmunoAssay) methods have been reported in the literature for ASFV diagnosis. The currently developed LFIA for ASFV are generally antibody detecting (serological tests), or not very sensitive (Ct<25), or suited only for blood or serum testing, not a convenient matrix for passive surveillance on dead animals. Here we describe the development of two rapid ASFV diagnostic tools: one visual LFIA detecting the p30 protein from ASFV and one based on RPA detecting the p72-encoding gene. A typical colorimetric LFIA test involves a visual readout. The LFIA was a sandwich-type immunoassay exploiting a monoclonal antibody (mAb) anchored onto the LFIA membrane to capture the ASFV and was also labelled with gold nanoparticles for staining the antibody-p30 complex. However, the use of the same antibody for capturing and as detector ligand showed a significant competitive effect for antigen binding, so required an experimental design to minimize reciprocal interference and maximize the response [5]. The new LFIA and RPA were applied for ASFV detection in the animal tissues usually analysed by conventional assays (i.e. real-time PCR), such as kidney, spleen, and lymph nodes from positive (from the Sardinian outbreak) and negative (from the hunting season archive in Piedmont) samples. A simple and universal virus extraction protocol was applied for sample preparation, followed by DNA extraction and purification for the RPA. The LFIA only required the addition of 3% H_2O_2 to limit matrix interference and prevent false positive results. The RPA assay, employing primers to the capsid protein p72 gene and an exonuclease III probe, was performed at 39°C for 25 minutes. The limit of detection of the method was assessed using a plasmid encoding the target gene and resulted in 5 copy/µL. The two rapid methods showed high diagnostic specificity (100%) and sensitivity (93% and 87% for LFIA and RPA, respectively) for samples with high viral load (Ct<27). False negative results were observed for samples with low viral load (Ct>28) and/or also containing specific antibodies to ASFV, which decreased antigen availability and were indicative of a chronic, poorly transmissible infection. The simple and rapid sample preparation and the diagnostic performance of the LFIA suggested its large practical applicability for POC diagnosis of ASF.

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P08

Three strategies for the monitoring of the immune response to SARS-CoV-2 based on Lateral Flow Immunoassay

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The implementation of the vaccination plans for COVID-19 is univocally assumed as the best strategy to control the spread of the infection and the return to economical ad social normalcy [1]. Unfortunately, there are some issues, such as supplying of the vaccine dosages or ingredients, organisation problems, etc…. This causes consistent delays to the reaching of the immunization of the population, and a stratification between different social classes. In addition, the appearance of more contagious variants of the virus implies the need to increase the ratio of vaccinated/not vaccinated population to limit diffusion and guarantee the full protection. Therefore, monitoring the efficacy of vaccination and follow the immune response of vaccinated and/or recovered people is very important in order to not nullify the efforts made in the vaccination campaigns. Highly accurate laboratory techniques cannot manage such large number of involved samples. Otherwise, antibody detecting point-of-care (POC) testing can fulfil this need. The lateral flow immunoassay (LFIA) is a widely employed POC test for COVID19 diagnosis, and serological format for antibody detection was employed in the very first phase of the pandemic [2]. A typical serological LFIA includes a nitrocellulose strip where capture immunoreagents are anchored in delimited reactive lines (test and control lines) and a glass fibre reservoir where the labelled detection reagent is dry stored. All these materials are partially overlayered embedded in a plastic cassette The serum is collected by a cellulose fibre pad, resuspend the labelled reagents and flows by capillarity through the nitrocellulose membrane encountering the reactive lines. Generally diagnostic tests are developed in order to increase indiscriminately the sensitivity, and serological LFIA were characterised by low sensitivity due to the delay in antibody response in infected people. Nevertheless, to follow up the immune response progression in time, more than a sensitive qualitative test, a quantitative correlation with the reference serological laboratory method (ELISA) should be preferred. In this work we developed three multitarget serological LFIA devices to detect antibody against the Spike protein and the Nucleocapsid protein from SARS-CoV2 (Figure 1) to discriminate the immune response given by recovery from infection and the one promoted by the vaccination. The devices were tested with 80 human sera dosed by ELISA, and the colour intensity of the test lines was quantitatively acquired. Correlation with reference ELISA was used as a figure of merit to define the best strategy for the follow up of the antibody response. The LFIAs for SARS-CoV2 antibody detection include capture antigens, recombinant Spike (S) and Nucleocapsid (N) proteins, and detection reagents, labelled with ruby red gold nanoparticles, different in the three formats (LFIA-1, LFIA-2, LFIA-3). In the LFIA-1 the recombinant antigens were labelled with AuNPs to promote a double antigen sandwich Ag*-Ab-Ag, to avoid random antibody saturation with double specificity. In the LFIA-2 staphyococcal protein A (SpA) is the detection reagent, to increase the sensitivity due to the 5 binding domains for the antibodies, with moderate preference for G class immunoglobulins (aka "immune memory" antibodies). The LFIA-3 employs secondary anti-human G immunoglobulins antibodies (anti-hIg) to detect selectively "immune memory" antibodies.

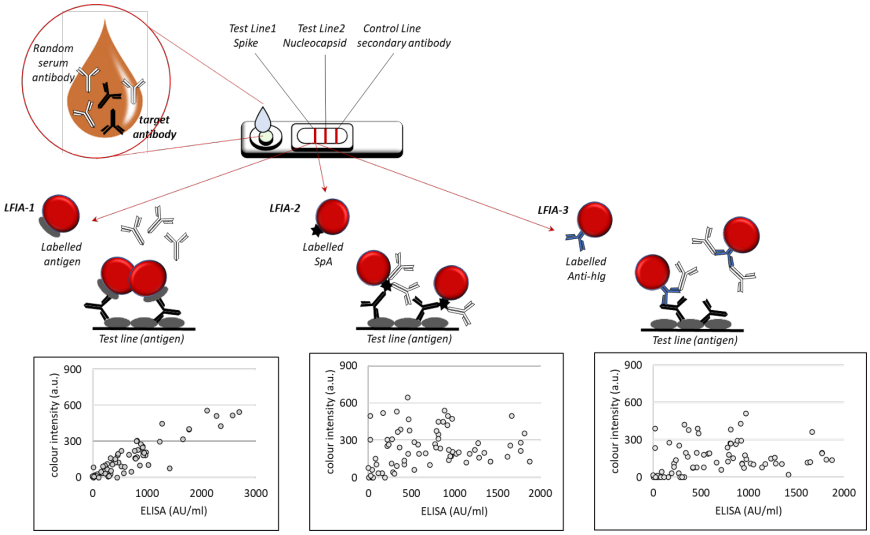

Figure 1: Schematic representation of the three formats of serological LFIA for antibody detection of SARS-CoV-2 immune response and correlation with reference serological ELISA method.

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P09

Solid-phase synthesis of nanoMIPs: the effect of delayed imprinting on the polymerisation

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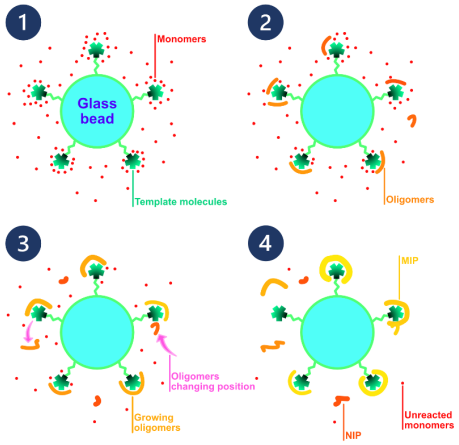
The solid phase polymerization synthesis (SPPS) is a versatile and innovative approach to obtain molecular imprinted nanopolymers with very high affinities and selectivities for the target molecules [1]. In this technique the template molecules are not free in the polymerization medium, but they are immobilized onto the surface of a solid support, usually glass beads. After the polymerization, nanoparticles are obtained with hydrodynamic diameters of 120/150 nm and with imprinted binding sites only on the surface and not in the bulk of polymeric structure. While the polymerization mechanism of the traditional MIP is well understood [2], and it is found on the formation of non-covalent interaction between the template and the monomers, and the subsequent "freezing" of the monomers position through the polymer cross-linking, the mechanism of the template imprinting in the SPPS is yet argument of debate. Rationally, there are no reasons why polymerization occurs only near the template molecules [3], but as polymerization starts at any point of the reaction environment with the formation of short oligomers, these can get in touch only later with the template molecules, rearranging around them. As the reaction proceeds, the complexity and rigidity of the particle progressively increases, making impossible for the growing oligomers rearrange around the template. As it has been demonstrated previously for traditional MIPs that oligomers are the key structures in the formation of imprinted binding sites [4], to better understand this mechanism, several nanoMIPs were synthetized introducing a delay between the beginning of the polymerization and the imprinting process. This was achieved by later addition of the glass-immobilized template – rabbit γ-globulins – in the reaction medium, after a fixed time after the beginning of the polymerization process. NanoMIPs were synthesized with delay times between 0 and 30 minutes, with an overall polymerization time of 60 minutes. For each nanoMIP the binding properties towards the template and a structural analogue – bovine γ-globulins – were determined by equilibrium rebinding experiments. The results show that delay times of 10 - 15 minutes cause a marked increase of the binding affinity and of the selectivity, while longer delay times cause an abrupt loss of affinity, confirming the correctness of the hypothesis of the influence of preformed oligomers on the formation of imprinted structures.



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P10

Evaluation of alkaloids content in *Lupinus albus* L. samples by means of HPLC-MS/MS

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Quinolizidine alkaloids (C_5NC_4 skeleton) are L-lysine derived compounds, having one or more nitrogen atoms usually contained in a heterocyclic ring system, which can be divided in bicyclic, tricyclic, and tetracyclic alkaloids[1].

These compounds are especially present in plants belonging to the *Lupinus L.* genus (Fabaceae family) and acting as a defense mechanism against pathogens and herbivorous animals. There are almost 70 different quinolizidine alkaloids found in various lupin species, which levels and combinations vary according to botanical and geographic origin, but also to soil composition and climate; they give bitter taste to the seeds and can cause even symptoms of poisoning in humans, affecting the nervous, circulatory and digestive systems. For this reason, bitter lupin seeds are not suitable for human or animal consumption without a proper debittering process[2].

According to this, the Regulations in Australia, New Zealand, France and Great Britain require compliance with a maximum level of 200 mg Kg^{-1} of alkaloids in lupin flours and in the seeds themselves[3].

There are few methods in the literature about the quantification of alkaloids in this specific food matrix; most of them reported the use of gas chromatography coupled to mass spectrometry (GC-MS), but recently there has been an increase of methods by high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) to detect alkaloid compounds in various matrices, both food and biological[2]. In this work, a sensitive method involving the use of HPLC-MS/MS was developed in targeted mode, with the aim of the simultaneous quantification and determination of different alkaloids. An efficient extraction procedure followed by a suitable clean-up step was also developed by Solid Phase Extraction, in order to decrease the amount of interfering compounds and to obtain reliable recoveries[4]. The presented method was fully validated following FDA guidelines and then applied to different batches of raw *Lupinus albus* L. samples originating from Abruzzo region, varying in size and farming treatments.

According to the analysis results, the different sizes and farming conditions appeared to have a significant influence on the alkaloids content in lupin samples; in particular, the less grown lupins showed a significant major concentration of alkaloids, as well as the biological farming samples showed an increased amount of alkaloids, due to the nitrogenate fertilizers. This information can be helpful for a farmer to understand what type of seeds should be selected for human consumption and, consequently, for the most appropriate debittering process.

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P11

Fluoroquinolones in human plasma and their UHPLC-PDA determination using electrospun sorbent for the solid-phase extraction

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In the last ten years, micro-extraction techniques or, alternatively, the application of new materials to already existing sample preparation technique have increasingly attracted the attention of the scientific community [1]. Particularly, in the field of adsorbents, electrospun fibers have had a great impact due to their unique physicochemical and mechanical properties and their huge chemically active surfaces. The electrospinning technique allows the production of ultrathin fibers with controlled diameter, size, morphology, and composition, charging the polymer solutions and directing them towards a collection manifold in a very homogeneous way. Briefly, in this technique, an electrical jet of spinning solution is produced by applying a high voltage between a needle and a collector. The micro-nanofiber is obtained when the repulsion of the surface charge of the solution exceeds its surface tension. Based on their morphology, nanofibers can be classified as core-shell, hollow, and porous material.

Fluoroquinolones were obtained starting from quinolones by adding a fluorine atom in position 6 and a piperazine in position 7 , giving the compound a different spectrum of activity . The first member of this series was the norfloxacin [2]. Fluroquinolones are analytes that have been extensively researched as a result there are numerous methods today for their determination and quantification [3].

Among sample preparation method used, the most used are those involving interaction with a sorbent, this is because solid-phase extraction or sorbent based methods offer greater reproducibility and because in recent years, more and more materials capable of offering performances clearly superior to those offered by traditional solvents in LLE have been developed. The prepared electrospun material, used as an sorbent material in SPE cartridges, was compared with the methods reported in literature.

An ultra-high performance liquid chromatography system (ACQUITY H-Class) equipped with sample manager, a quaternary pump, a column heater and a photodiode array detector was used for the FLQ separation and quantification. Chromatographic separation was achieved using a CORTECS C_{18} Column (75 x 2.1 mm, 2.7 μm). The mobile phase consisted of 10mM ammonium acetate adjusted at pH 4 (phase A) anda mixture of acetonitrile-methanol (80:20 v/v), phase B, both phases were added with 0.1% of triethylamine.

In this study, electrospun PAN PMMA membranes optimized in a 5:1 ratio were studied and characterized for the realization of sorbents for the extraction of fluoroquinolones in plasma samples.

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P12

Lateral flow assays based on decorated DNA scaffolds

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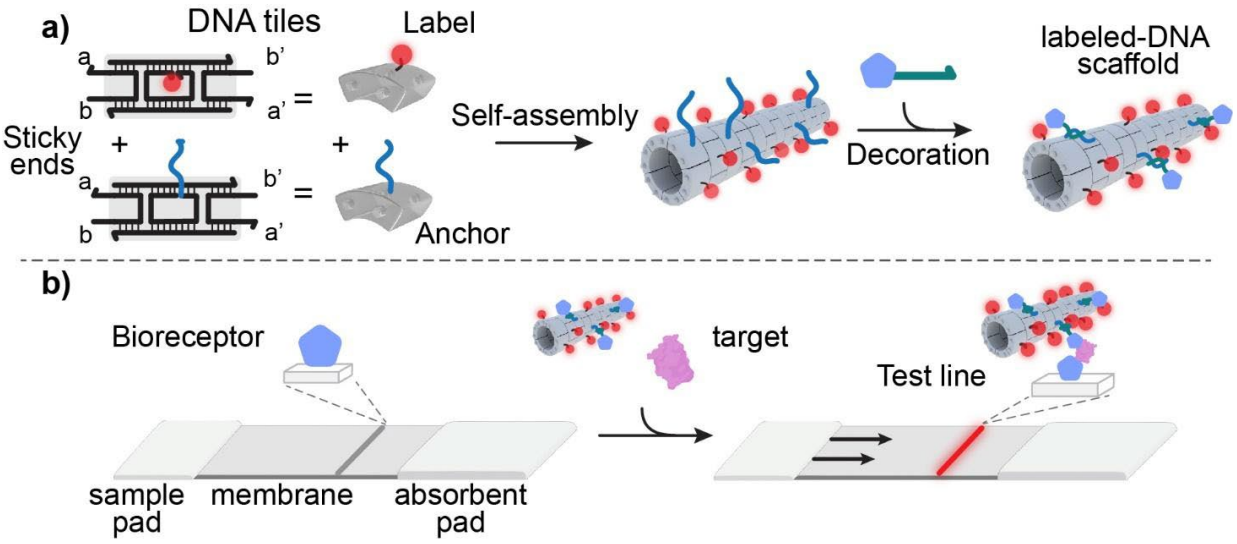
In the last decades significant advances in the development of lateral flow assays have been achieved. Numerous examples for the detection of antibodies, viruses and bacteria, with high sensitivity and specificity, have been described. Despite this, new approaches to create lateral flow platforms with increasing versatility and applicability are needed. DNA nanotechnology allows the design of devices, systems and structures that can be engineered with nanometric precision. Thanks to the high programmability and specificity of non-covalent hydrogen bond base pairings, synthetic nucleic acid strands (i.e DNA) have emerged as ideal materials that can find application in a variety of fields, such as diagnostic, sensing, bioimaging and drug delivery.

In response to this, we show a novel strategy to build lateral flow assays that employ decorated DNA nanostructures as functional scaffolds to generate a readout signal upon the recognition of specific targets. The use of functional DNA scaffolds coupled with lateral flow assays allows to reach several advantages. First, the assembly of DNA structures displaying functional groups of different nature (i.e. small molecules and fluorophores) allows to employ them to generate a fluorescent readout signal only upon the recognition of the specific target. Second, the possibility to introduce different recognition elements enables the detection of diagnostic targets, ranging from antibodies to small molecules or proteins. Finally, we show the possibility to develop an assay for the simultaneous detection of multiple targets.



P13

One-step ELIMC-based assay for detection of total Microcystins and Nodularins in water samples: setting-up of the experimental conditions for modulating sensitivity

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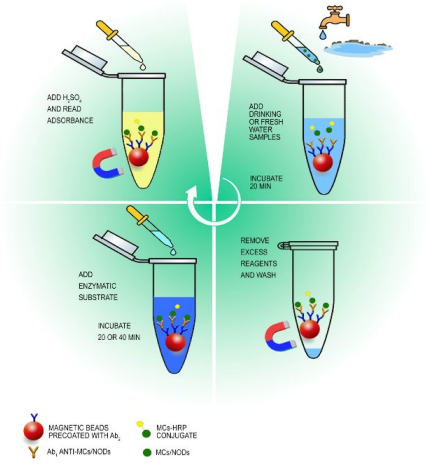
Microcystins (MCs) and Nodilarins (NODs) are secondary metabolites produced by cyanobacterial blooms in fresh and brackish water worldwide. Given the harmful effects of these compounds on consumers' health, Italy was one of the first European countries to adopt a regulatory value of 1 µg/L for the total content of MCs in drinking water. Since in 2021 MCs and NODs have been included in the list of drinking water contaminant candidates by the U.S. EPA [1], the monitoring of both toxins, by using cost-effective, reliable and sensitive detection methods, is desirable.
Herein we describe the set-up of a one-step sensitivity-modulable ELIMC (Enzyme-Linked Immuno-Magnetic Colorimetric) assay for the determination of microcystins/nodularins in water samples.
The first part of the work was devoted to revisiting a commercial ELISA Microcystins/Nodularins tube kit using magnetic beads as simple and effective support for the immunological chain. This kit was carefully selected, among those commercially available, for its intrinsic characteristics such as simplicity, rapidity, and relatively moderate cost. A comprehensive comparison between the ELIMC assay and the ELISA tube kit was carried out. Our assay showed a detection limit, sensitivity, working range and rapidity similar to those of the commercial kit but, thanks to the use of MBs, the analysis number was increased from 40 to 250. Satisfactory recovery and precision, obtained by analyzing controls, drinking and freshwater samples, proved the suitability of the assay when the screening of numerous samples is required for regulatory and environmental monitoring purposes.
In the second part of the work, we focused on enhancing the analytical performances of the ELIMC assay by making simple changes to the protocol, thus avoiding the need for more sensitive, complex, and expensive ELISA kits for quantifying very low amounts of MCs/NODs. By doubling the enzyme-substrate reaction time and halving the concentration of the primary antibody, a lower detection limit (0.03 ng/mL), an increased sensitivity (0.26 ng/mL) and a wider working range (0.05-2.0 ng/mL) were reached. Recovery from 84 to 120% and precision (RSD) from 7 to 16% demonstrated the reliability of the enhanced ELIMC assay to measure very low concentrations of MCs/NODs in water samples, allowing the adoption of timely corrective actions in the frame of environmental recovery programs.



Schematic representation of the ELIMC assay principle

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P14

Evaluation of the alterations in mice urinary metabolomic profile following the administration of opioids by UHPLC-HRMS

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The proliferation of new psychoactive substances (NPS) and the challenges associated with their detection have driven forensic toxicologists to identify suitable analytical strategies for their identification in seizures and biological samples. Untargeted metabolomics was identified as an useful approach to identify endogenous markers of NPS assumption. In this study, this tool was exploited for the identification of biomarkers that may indicate the probable intake of opioids of different chemical classes - by exploiting an in-vivo study in mice and HPLC-HRMS analyses.

Urine samples were collected from CD-1 mice, both males (n=8) and females (n=8).; All animals were initially treated with 0.9 M NaCl-containing saline (vehicle) by intraperitoneal injection; urine was collected cumulatively over 24h. Subsequently, half of the animals of both sex were treated with 30 mg/kg morphine while 6 mg/kg fentanyl was administered to the rest of the animals; then 24h urine was also collected. Samples were diluted prior to UHPLC-HRMS analysis. Mass spectra were acquired on an Orbitrap Q-Exactive mass spectrometer equipped with a HESI source; every sample was analyzed with both RP and HILIC chromatography in both polarities. Univariate and multivariate analysis was carried out on the data obtained.

The results obtained from the OPLS-DA (the number of variables was reduced until the best Q2 value through the application of successive OPLS-DA models), led us to identify the main differences, in terms of metabolites, after drug administration; for example 5-aminovaleric acid and creatine and others belonging to the main lipid oxidation and amino acids degradation pathways were among the most altered metabolites. In general, the literature can only offer a limited number of studies on the urinary metabolic profile of opiate-treated rats. For each sample, in order to eliminate inter-individual variability, basal metabolic profiles (those obtained from vehicle administration) were subtracted from those given by administration. Similarly, a multivariate analysis was performed in order to elucidate differences related to the sex of the animals.

The study investigated alterations in metabolic pathways of CD-1 mice treated with opiates; it highlighted the potential of metabolomics in forensic toxicology for investigations related to NPS.

P15

Exploiting platinum nanoparticles as colorimetric label in Lateral Flow Immunoassay

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The lateral flow immunoassay (LFIA) has resolutely become one of the leading bioanalytical techniques for point-of-care (POC) and point-of-need (PON) analyses. These tests consist of a plastic support coated with a nitrocellulose layer and other porous materials overlapping each other, allowing the liquid sample to move through capillarity and encounter specific reagents for detecting the target substance. LFIAs allow to perform analyses at the sampling site usually exploiting the immunoreactions between an antibody and a target antigen and converting this response into a measurable signal thanks to the use of proper labels (visually detectable signal in its simplest configuration). The results are obtained in a timely manner (usually within 5-30 min), in an easy way (simply requiring the addition of the sample to the test device) and requiring minimal equipment and training; moreover, LFIA offers all these advantages at a low cost [1]. LFIA tests are highly sensitive and specific and have been applied in a wide range of fields, including human health, veterinary medicine, food safety, environmental monitoring, forensic analysis, and many others [1].

Currently, the most used label in LFIA to obtain visually detectable signals are gold nanoparticles (AuNPs) due to their competitive advantages even regarding the analytical performances [1]. Nevertheless, in recent years, significant efforts have been made to enhance the lateral flow immunoassay technique, with a focus on improving its sensitivity [2].

Most of the suggested strategies to enhance the analytical performances includes additional steps, additional chemicals and different detection methods that rely on the use of detectors or readers making the analysis more complicated, expensive, and time-consuming.

To reduce complexity, costs and time per analysis, some efforts have been also dedicated to exploring the use of new colorimetric labels, with the aim of providing a valid alternative to the AuNPs use, as we can appreciate the recent literature [3,4].

Among the colorimetric labels, platinum nanoparticles (PtNPs) might be a good candidate to be used in LFIA development considering their interesting optical properties [5].

In this work, a protocol for PtNPs synthesis was established as well as a conjugation procedure between specific antibodies and PtNPs. Finally, PtNPs have been successfully used to develop a LFIA for the detection of two mycotoxins, i.e., aflatoxin B1 and Fumonisin B1, showing their effective use as alternative label.

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P16

Sustainable, Cost-Effective, and Flexible Screen-Printed Potentiometric Sensor for Reliable Ion Chloride Detection in Sweat

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Nowadays, the most relied-upon analytical methods are still based on well-equipped laboratories,requiring bulky and expensive instruments, skilled personnel, and lengthy detection processes. With the increasing need for overcoming these drawbacks, the researchers' attention has turned to easy-to-use miniaturized analytical devices for point-of-care (POC) testing. In this regard, electrochemical sensors based on a screen-printed electrodes (SPE) have been extensively used in analytical chemistry due to their easiness of usage, flexibility, portability, sustainability, and excellent electrochemical performances for multifarious analytical applications. In this context, the development of innovative sensing approaches for biomarkers in biofluids, e.g., saliva, tears, and sweat, pave the way for advantageous solutions to the monitoring and diagnosis in the healthcare field.

Among these new approach's, wearable sensors can give crucial information regarding a wearer's health in real time due to their biocompatibility and lightweight on the skin. By offering reliable and user friendly real time data acquisition, wearable sensors allow individuals to change their lifestyle in order to maintain optimal health status leading to a major shifts from hospital based patient care to home based personal management and then a decrease in healthcare costs. Sweat, for example, is a noninvasively acquired bio-fluid that contains several electrolytes such as sodium, potassium, and chloride, providing information on the health state [1]. Indeed, sweat components can be used as biomarkers for a variety of diseases as well as to measure electrolyte balance and hydration status. Among these biomarkers, chloride is one of the most important, as it is involved in different physiological functions, including the maintenance of osmotic pressure, the movement of water across membranes in fluid compartments, muscular activity, and electron neutrality [2]. Therefore, there is an urgent need for chloride monitoring in real-time. In this context, we developed a miniaturized wearable sensor for Cl- monitoring in sweat during physical activity. The designed platform consists of a low-cost fabricated potentiometric sensor, combined with a miniaturized potentiostat bluetooth for data transmission. In order to create a layer sensitive to the different ions, the working electrode surface was modified with 6 µL of carbon black, and ion-selective membrane containing chloride ionophore I. The reference electrode was modified with polyvinyl butyral-based membrane to maintain a stable potential in solutions with different ionic strengths.

Firstly, several experimental parameters were studied using bench potentiostat, in terms of volume and conditioning of the reference and ion-selective membranes, interferences study, and pre-treatment procedure for stability enhancement. The calibration curve was obtained by potentiometric measurements in standard solutions containing Clin the concentration range between 10-3 to 1 M, described by the following equation: $y = (-0.060 \pm 0.002)\ x + (0.418 \pm 0.005)$. Finally, the applicability of the wearable device was tested by measuring Clin real sweat, sampled during physical activity. In the next step, the suitability of the developed wearable platform will be evaluated by Cl- monitoring in real time and in non-invasive way in order to give rise to a wearable sensor able to detect chloride ions for healthcare monitoring.

Keywords: Ion-selective membrane, Carbon black, Potentiometric, Screen-printed, Sweat chloride, Wearable sensor.

P17

Design and optimization of an on-fiber chemiluminescence biosensing system

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The aim of the present work is the preliminary investigation of a platform for the optimal implementation of chemiluminescence (CL) based biosensing.

Among all the optical strategies for biosensing, CL offers many positive features, such as high sensitivity of detection even in low volumes, no need for any external light source and simple instrumentation required for its measurement, which make it particularly suited for the development of ultrasensitive assays in a portable format for point-of-care (POC) settings. Nevertheless, the analytical performance of most of these portable devices is mainly limited by an inefficient optical coupling between the biosensor compartment where photon emission occurs (e.g., microfluidic chip) and the photodetector.

The common approach in the implementation of a CL based detection system is to have the CL reaction and consequent emission of photons from bulk solution inside an assay vial, and to collect the light by "looking at" the vial content. The main idea of this work is to "force" the CL reaction in the close proximity of an optical waveguide that safely contains and guides the generated light up to the detector. In this sense an optical fiber matches perfectly the required properties. The challenging idea is to make the reaction occur directly on (very close to) the external lateral surface of the fiber core. The radiation emission of a dipole in the proximity of a dielectric interface (water environment – fiber silica/glass/plastic core) happens mainly in the material with higher refractive index (RI), the fiber core, and with a slope that allows the guiding by total internal reflection. The optical fiber cannot be used as it is, but must be strongly modified to avoid the loss of optical signal: the core must be exposed in the reaction region, the numerical aperture of the fiber must be the higher as possible, possibly the cladding and coating must be removed in the whole length of the fiber.

The aim of this work is the realization of an optimized optical fiber CL biosensing system in which the reaction occurs very close to the external lateral surface of an optical fiber core.

Preliminary data were obtained by a modified multimode fiber inserted in a capillary used to monitor a CL signal generated after an ovalbumin immunoassay.

Acknowledgments. This work was supported by the Italian Ministry for University and Research in the framework of "Bando PRIN 2017", project number 2017YER72K.

P18

Smart sensor for lithium drug monitoring detection

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Lithium is a naturally occurring chemical element used as a psychiatric medication. In particular, lithium salts are widely employed in the treatment of several mental illnesses, such as bipolar disorder, schizophrenic disorders or depression. However, lithium has a very narrow therapeutic range (0.8-1.5 mM) and exceeding the limits could cause several irreversible side effects and could lead, in more severe cases, to death. The quantification of lithium requires frequent blood tests resulting in pain and discomfort in patients, which turns in evading treatment [1]. In this scenario, the importance of developing non-invasive analysis techniques, which are able both to detect lithium ions with high sensitivity and replacing blood with other painless collectible body fluids, becomes evident.

Electrochemical sensors like ion-selective electrodes (ISEs) are a promising alternative to most of the existing analytical techniques for an accurate lithium detection through painless analysis [2]. In fact, thanks to the advancement of smart technology the possibility of obtaining miniaturized analytical devices for on-body drug monitoring is highlighted

In this work, we propose a smart solid-contact sensor for lithium detection in synthetic human samples. Disposable screen-printed electrodes (SPEs) were used to develop a performing device. In particular, graphite screen-printed electrodes were modified by electrodepositing silver and gold nanoparticles ((Au&Ag)NPs@GSPEs), and then by drop-casting an ion-selective membrane. The developed sensor was used in drop and microflow configuration and showed a high sensitivity for lithium with a low detection limit (1.6 μM) [3]. The proposed sensor was connected to a smartphone thanks to the use of a miniaturized potentiometer. The applicability of the platform for wearable implementation was assessed by the determination of lithium in different human body fluids, in particular sweat and saliva.

The developed sensor was tested in synthetic human saliva and sweat samples showing a linearity in the range of 10^{-4} – 10^{-1} M with a LOD of 0.4 mM and 1.5 mM in sweat and saliva samples, respectively. This confirmed the possibility to use the platform as an integrated wearable monitoring system.

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P19

An impedimetric approach to detect β-lactoglobulin in milk-free products

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β-lactoglobulin (β-LG) is the most common source of milk allergy because of its remarkable stability and its absence in human milk; moreover, it is also regularly added as an additive in lots of food products [1,2]. Food allergic patients are still at high risk of unintentionally consuming trace amounts of allergens that may have contaminated food products during the production line. In this perspective, it becomes clear that to establish an appropriate analytical methodology for detection of traces of allergens is of outstanding importance; moreover, the investigation on the effect of food processing on both allergenicity and detectability needs to be carried out.

Electrochemical aptasensors are characterized by a high specificity and sensitivity thanks to the affinity reaction between the aptamer and the analyte, even in complex matrices. However, key steps in their development are represented by the immobilization of the aptamer and the signal generation. Therefore, platforms with overall improved electrochemical features that can immobilize the aptamer probe in a tailored manner to enable the optimum signal generation are of high demand [3].

In this work, an electrochemical aptasensor for β-LG detection based on a label-free impedimetric approach was realized. Disposable graphite screen-printed electrodes (GSPEs) were used to develop a performing device that could be potentially applied for on-site analysis. The conjugation of a polymeric matrix constituted of poly-L-aspartic acid (pAsp) [4] with nickel nanoparticles (NiNPs) [5] was exploited for the ultra-sensitive detection of β-LG. The building steps involved in the realization of the aptasensor were all investigated and optimized by electrochemical techniques, such as cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The aptasensor was integrated in a smart setup comprising a pocket instrument connected to a smartphone. The applicability of the platform was assessed by determining β-LG in different kind of vegetal milk samples.

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P20

Innovative combination of thermal desorption with on-line solid phase extraction reversed phase liquid chromatography applied to targeted nutrimetabolomics in human biofluids

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This study proposed for the first time the use of thermal desorption in the on-line solid phase extraction coupled with reversed phase liquid chromatography (on-line SPE-RPLC) using mixed mode polymeric sorbents. This analytical strategy was applied to the on-line SPE-RP-LC targeted analysis of a model set of 34 human gut metabolites characterized by heterogeneous physicochemical properties (e.g., octanol–water partition coefficient in the range -0.3 – 3.4). The novel temperature-assisted on-line SPE approach was investigated in comparison with the conventional desorption strategies at ambient temperature based on the use of (i) an optimized elution gradient or (ii) an organic desorption followed by post-column dilution[1]. The thermal desorption strategy has been shown to be better performing and suitable for the development of a reliable and sensitive method for the analysis of the model group of analytes in urine and serum. In more detail, under the optimized experimental conditions (Isolute ENV+ sorbent, desorption temperature 120°C, desorption time 5.5 min), the proposed method provided negligible matrix effects in both biofluids for almost all target analytes. Moreover, method quantification limits were in the ranges 0.026-7.2 μg L^{-1} and 0.033- 23 μg L^{-1} for urine and serum, respectively, i.e., comparable to or lower than those reported in methods previously published[2,3].

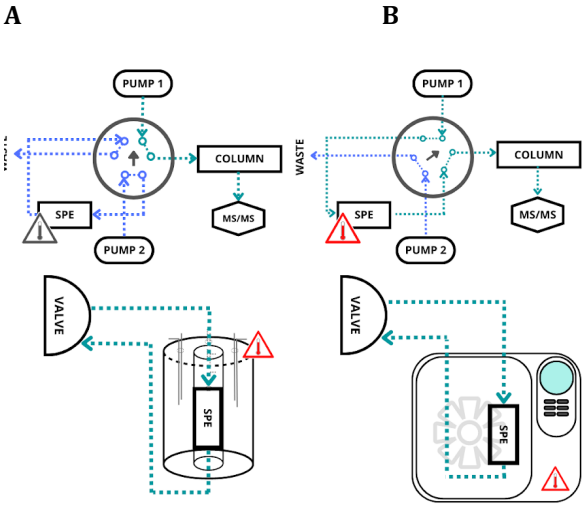

Figure 1 – Schematic illustration of the thermal desorption systems 1 (A) and 2 (B)

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P21

AF4 coupled to chemometrics for the analysis of protein fraction of milk

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In this work, the Asymmetric Flow Field Flow Fractionation (AF4) analysis was applied to the characterization of the colloidal fraction of milk samples. By AF4, it was possible to evaluate the protein fraction of milk with the aim of discriminating samples based on the production method, geographical origin, and production manufacturer.

Forty-nine commercial milk samples were considered; thirty-seven of them were produced by the Ultra High Temperature (UHT) procedure, and twelve were not-UHT. For all of them the geographical origin and the manufacturing plant were known. The discrimination was performed using Principal Component Analysis (PCA), applied to the full fractograms.

By evaluating PCA scores plot, as a first result, it was possible to discriminate UHT and not-UHT milks, as reported in Figure 1: two well-discriminated clusters are present, the red one composed of UHT samples, and the blue one, the not-UHT samples. [1]

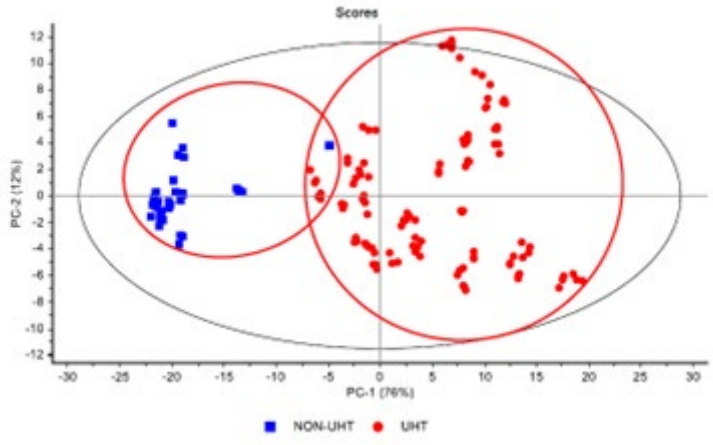

Figure 1 scores plot of UHT and not UHT milk samples

A second PCA model was calculated for the UHT milk samples only. Such model was able to discriminate samples based on their geographical origin and producer. The geographical origin discrimination was less strong than the producer plant one, because it is known that milk can be transported also for long distances before being bottled. Therefore, the effect of the treatment to which the milk is subjected to in the production plant covers the information derived from its geographical origin,

This preliminary work underlines how different production methods influence the protein content of milk, and shows that AF4 is a very suitable method for protein separation and characterization. Coupling this separative technique with chemometrics, it was possible to use the protein fingerprint of milk samples to evaluate the differences due to production method (UHT and not-UHT) and to the origin of the product.

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P22

A new microsampling and enantioselective liquid chromatography coupled to mass spectrometry approach for the bioanalysis of novel psychoactive substances

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In this work, the development of efficient enantioselective HPLC methods for the analysis of five benzofuran-substituted phenethylamines, two substituted tryptamines, and three substituted cathinones was realized. For the first time, MS compatible RP eluents made up with acidic water-MeOH solutions, as well as PI eluents carrying an ACN-MeOH solution with both an acidic and a basic additive were employed in combination with a CSP incorporating the (+)-(18-crown-6)–tetracarboxylic acid chiral selector. Enantioresolution was achieved for nine compounds with α and R_s factors up to 1.32 and 5.12, respectively.
Circular dichroism (CD) detection, CD spectroscopy in stopped-flow mode and quantum mechanical (QM) calculations were successfully applied to establish a (*R*)<(*S*) enantiomeric elution order of mephedrone, methylone and butylone under the optimized elution conditions.
Whole blood miniaturized samples collected by means of volumetric absorptive microsampling (VAMS) technology and fortified with the target analytes were extracted following an optimized protocol and effectively analysed by means of an UHPLC-MS system. By this way the suitability of the method for quali-quantitative enantioselective assessment of the selected psychoactive substances in advanced biological microsamples was clearly demonstrated. VAMS microsamplers including a polypropylene handle topped with a small tip of a polymeric porous material were used and allowed to volumetrically collect small aliquots of whole blood (10 µL) independently from its density. Highly appreciable volumetric accuracy (bias, in the -8.7-8.1% range) and precision (% CV, in the 2.8-5.9% range) turned out.

P23

A Guanosine-Derived Supramolecular Hydrogel with DNAzyme-like peroxidase activity as a novel platform for hydrogen peroxide detection

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Chemiluminescence (CL) is a widely used method for detecting hydrogen peroxide (H_2O_2), due to its high sensitivity, ease of use, and low cost. The most widely employed system is based on horseradish peroxidase (HRP) and luminol. H_2O_2 plays an important role in various applications and is a crucial biomarker in monitoring various diseases and disorders, including diabetes, cancer, Parkinson's disease, Alzheimer's disease, cardiovascular and neurodegenerative disorders. Finally, H_2O_2 is also produced in reactions catalyzed by numerous oxidases, such as glucose oxidase, alcohol oxidase, cholesterol oxidase, lactate oxidase, and glutamate oxidase [1].

Hydrogels have gained great interest in the development of biosensors, owing to their high biocompatibility and the ability to incorporate foreign substances while preserving a benign environment for the biosensing events [2].

The 3D porous structure of hydrogels increases the surface area of the material, allowing the loading of large amounts of recognition elements (ranging from small molecules to proteins and even cells), better accessibility and a more biocompatible environment provided by the flexible and aqueous nature of hydrogels. The preservation of the native structure of biomolecules is a crucial requirement for feasibility, specificity, and sensitivity in biosensing applications [2].

A chemiluminescence (CL) hydrogel was prepared by simultaneous incorporation of a CL reagent (luminol) and catalytic cofactor (hemin) into the scaffold of a guanosine-derived hydrogel. The self-assembled hydrogel consisted of entangled K^+-stabilized hemin/G-quadruplex structures, showing significant DNAzyme-like peroxidase activity to H_2O_2-mediated oxidation of luminol (Fig 1A) [3]. After adding H_2O_2 into the hydrogel, the CL light signal is generated and lasts for several minutes. The long-lasting CL emission of hydrogel was achieved due to a mechanism of slow-diffusion-controlled heterogeneous catalysis. Under optimal conditions, the CL increases linearly from 0.05 mM to 1 mM for H_2O_2 with a limit of detection (LOD) of 0.05 mM (Fig 1B). This soft biomaterial can also be used for the indirect detection of glucose, upon incorporation into the hydrogel of glucose oxidase, which produces H_2O_2 upon oxidation of glucose. A calibration curve has been constructed obtaining a LOD of 0.2 mM and a good selectivity of the system. As proof of concept for the development of a biosensor that uses hydrogel as a matrix for glucose detection, the CL signal has been acquired also via smartphone, using a 3D printed dark box.

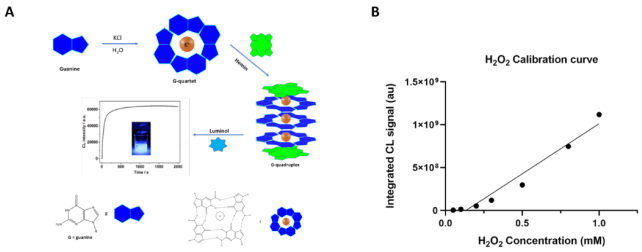

Figure 1. Scheme of supramolecular self-assembly of guanosines in the presence of K^+, hemin and luminol (A); H_2O_2 calibration curve (B).

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P24

The bioluminescent recombinant protein Jagged1-FLuc as potential diagnostic tool for the high- throughput screening of colorectal cancer

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Colorectal cancer (CRC) ranks as the third most common tumor in the western world in men and the second in women, with about 43,700 new diagnoses in Italy in 2020 and about 21,700 deaths in 2021 [1]. These data are unacceptable, considering that CRC has a substantially favorable prognosis if diagnosed in the early stages, therefore the discovery of new predictive biomarkers is highly demanded. Recent studies suggested a pivotal role of "aberrant" Notch signaling activation in CRC, partially due to cross-talks with other pathways such as Wnt and Erk/MAPK. In this context, we developed a bioluminescent recombinant protein using the Baculovirus expression system in insect cells. Specifically, the protein combines the extracellular domain (ECD) of the Notch high affinity mutated form [2] of the selective Notch ligand Jagged 1 (Jag1) with a Red emitting firefly luciferase, widely used for bioluminescence imaging (BLI) in cells or tissue, in order to understand if Jag1-FLuc binding correlates with Notch overexpression in CRC progression. Firstly, we tested the new construct in a cell-free system to evaluate the protein's light output. Kinetic profiles of bioluminescence (BL) emission were analyzed triggering the reaction after the addition of the substrate solution (luciferin, Mg^{2+}, and ATP) in the presence of different concentrations of the protein (range: 0.25 -10.0 μg/mL) thus obtaining a good linear correlation between the BL signal and the concentration of Jag1-FLuc protein up to 10 μg/mL of the protein, with a LOD and LOQ of 0.20 ± 0.03 and 0.50 ± 0.03 μg/mL respectively. Once the method was optimized in a cell-free system we moved on to a cell model using the human colorectal adenocarcinoma cell line Caco-2 which to our knowledge expresses high levels of the Notch receptor 3 in plasma membranes. Under the optimized conditions, in Caco-2 cells ($1x10^5$ cells/well) we observed that BL signal increase was proportional to the Notch 3 expression, with a linear range from 0.1 to 50 μg/mL of Jag1-FLuc obtaining a LOD and LOQ of 0.8 ± 0.2 and 6.0 ± 0.2 μg /mL, respectively. To confirm that Jag1-Fluc didn't bind to the cells in a non-specific way the probe selectivity was checked using a soluble Jag1-IgG1 Fc chimera as a competitor, both on Caco-2 cells and also on MCF-7, a breast cancer cell line that expressed high levels of all Notch receptor isoforms. Fixed cells were firstly incubated with increased concentrations of the human soluble Jag1 chimera in the range of 0.1-25 μg/mL overnight and then Jag1-Fluc was added to reach a concentration of 50 μg/mL, then the incubation was prolonged for 8 hours. After two PBS washes and the substrate addition, we observed that the BL signal decreased proportionally to the concentrations of the competitor, obtaining an IC50 of 0.55 ± 0.06 μg/mL and 0.45 ± 0.04 μg/mL in Caco-2 and MCF-7, respectively. Once confirmed the probe selectivity versus Notch receptors, imaging experiments were performed to examine the light output of Jag1-FLuc construct on Caco-2 cells and *ex vivo* on human biopsies derived from subjects with different intestinal tumor lesions (hyperplastic polyps, low- and high-grade adenomas, and cancer lesions). In each of these models, we successfully demonstrated the possible application of the bioluminescent Jag1-FLuc protein both for quantification and imaging of Notch expression. Thus, Jag1-FLuc could represent an important new tool to improve the early detection and diagnosis of pre-neoplastic and neoplastic lesions. The bioassay is simple and at the same time very sensitive. Finally, the identification and validation of the Notch signature, potentially associated with a high risk of colon cancer progression, could suggest a wide range of clinically relevant applications, including new screening and therapeutic approaches for CRC.

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This work was supported by the PRIN 2017 (Prot. 2017Y2PAB8) and Fondazione Cassa di Risparmio di Bologna (ID 19045- COD SME 2020.0399).

P25

Chemical characterization of a deep Ionian Sea sediment core

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The environmental Trace Elements (TEs) contamination is one of the main problems related to both acute and chronic pollution phenomena due to human activity, which can generate several disturbances in the ecosystem. In fact, some TEs are persistent pollutants that are difficult to remove by natural recovery processes and tends to accumulate in sediments[1]. In the sediments it is possible to distinguish two environments: a superficial one, directly affected by the actual water-column state and a deeper one, "undisturbed", which can contain important information about the historical contamination of the area. During the 2006 ISMAR-CNR oceanographic campaign CADI, a deep Ionian Sea (M5, 4017 m depth, Fig. 1) sediment core was collected by using a gravity corer in order to better understand the sedimentological and anthropogenic processes in the deep Ionian Sea environment. The core was extruded on board to obtain 18 subsamples for the further analysis of elements, Total Carbon (TC), Total Organic Carbon (TOC), Total Inorganic Carbon (TIC), grain size, pH and Eh. Elements, such as Fe, Mn, and Zn, were analysed by Flame Atomic Absorption Spectroscopy (FAAS), Al, As, Cd, Cr, Ni and Pb by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) after acid microwave assisted digestion, while total Hg was determined by Direct Mercury Analyser. A change of the sediment nature was observed from the 20-30 cm depth to the bottom of the sediment core, highlighted by the different variation of the carbon fractions with the depth (TOC decrease and TIC increase). This resulted in a sharp division between the mostly Clay and Organic surface layers and the deeper layers of the core where carbonates were dominants.
Preliminary results on element concentrations allowed to identify three vertical distribution patterns in the sediment core. Al, Cr, Hg, Pb, Fe, and Zn showed generally higher concentrations in the surface layers and a decreasing pattern with the core depth. This trend is associated with a decrease of Al and TOC content. Instead, As and Mn showed a maximum at 25-40 cm of the core, while Cd followed a homogeneous trend. An interesting opposite variation between Fe diminution and Mn increment was observed between the 20 and 30-cm depth of the core, suggesting the occurrence of early diagenesis processes. Spearman correlation matrix (Figure 2) showed a negative correlation between TC and Al, As, Cr, Fe, Zn, while positive significant correlations were observed for TOC with Hg and Pb and for Al with Fe, Cr, Pb and Zn. The statistical analysis confirms the different composition of the surface and the bottom layers of the sediment core. The Enrichment Factors (EFs) and geoaccumulation index (I_{geo}) calculated for each elements suggest natural crustal sources for all the elements, except for Ni and Cr. The comparison of the average metal concentrations with the Environmental Quality Standards for sediments (EQS) according to US EPA and Directive 2008/105/EC[2], showed moderate pollution for Cr and Ni, while As exceeded by about 40% the EQS.

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P26

Exploring the diagnostic potential of chemometric fingerprinting from FFF-sorted biological samples to track and detect exosomal population in cancer patients' serum

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There is a growing interest raised by extracellular vesicles (EVs) both as diagnostic analytes and therapeutic vessels. Consequently, many techniques and approaches are exploited to collect EVs from various sources (cell media or human blood serum, saliva and other body fluids) in order to explore the potentialities of non-invasive liquid biopsies, improve diagnosis, and understand the biological function and synergistic action on EVs in cellular communication.

The drawbacks of these approaches reside in the many steps required for EV isolation, the scarce throughput, and the complexity of EV characterization, by means of labelling, mass spectrometry and immunoassays (ELISA). Field flow fractionation (FFF), a soft separation technique able to work in physiological conditions and sort particles from complex samples according to hydrodynamic size, was previously employed to successfully isolate EVs from cell culture medium [1], though a pre-concentration step was required. More recent advancements of our FFF-based EV isolation protocol allowed to also process biological samples such as human blood serum without any pretreatment (e.g. ultrafiltration): this is a crucial advancement in EV research since it would cut down cost and limit sample manipulation. Preliminary results showed that it is possible, through FFF separation, to collect EV-enriched fractions to undergo ELISA characterization. This work, involving samples from patients with colorectal cancer (CRC), put the basis for the fast isolation and identification of diagnostic markers avoiding invasive procedures.

The advantages of using an FFF platform to isolate EVs also include the chance of characterizing the samples by the means of online detection prior to fraction collection, obtaining multidimensional information which contains UV absorption and fluorescence signals relative to the species eluted during the process, together with mass/size characterization obtained by multiangle light scattering.

With the aim of exploring the potential of such information, a chemometric approach based on visualization and classification techniques was carried out.

The feasibility study tackled the complexity of the data sets obtained and pointed out the useful descriptors for the different EV. At the same time useful information to forecast and direct EV enrichment for maximizing information from the sample and reducing invasive medical procedures, were obtained.

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P27

Untargeted metabolomics strategy based on ultra-high-performance liquid chromatography–high-resolution-ion mobility-mass spectrometry for cereal quality and traceability

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Metabolomics has grown into one of the major approaches for systems biology studies, in part driven by developments in mass spectrometry (MS), providing coverage of the metabolome at high throughput. Concerning plant metabolomics, MS-based untargeted methods can be regarded as a valuable tool to fill the knowledge gap regarding the effects of agro-sustainable treatments on food quality in terms of nutritional content and technological properties[1,2].

In the present study, an ultra-high performance liquid chromatography/high resolution ion mobility-mass spectrometry (UHPLC-HR-IM-MS)-based strategy was devised to get insights into the effects of sustainable agro-processes on the metabolic pattern in durum wheat cultivar Svevo grain, one of the most important cereal crops of Mediterranean region, grown in 2020 and 2021. In particular, UHPLC-HRMS analysis using a Synapt-G2-Si HDMS hybrid quadrupole time of flight mass spectrometer in both positive and negative modes enabled to acquire a comprehensive metabolite profiling of wheat grain associated to four treatments, i.e., control, biochar addition, commercial biostimulant addition and the treatment with biochar combined with commercial biostimulant. With the aim to identify and elucidate biomarkers able to differentiate among the different treatments, the prioritization of the features from raw data acquired by MS^E mode[3] involved the calculation of the fold changes in the signal intensities and supervised multivariate statistical analysis. Partial least squares discriminant analysis (variable importance in the projection-VIP- > 2) allowed to select more than 130 and 170 VIPs for Svevo 2020 and Svevo 2021, respectively. Then, metabolites were identified based on available public databases. Collision cross section values were used as valuable additional identification criteria for metabolite elucidation. Finally, k-fold cross validation proved the reliability of the selected variables in discriminating the treatments.

The results achieved in this study will pave the way to the use of untargeted metabolomics to assess and improve quality and traceability of cereals, including their beneficial effects on health, and for the establishment of harmonized conditions for making fertilizers made from recycled or organic materials available on the market.

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Acknowledgments: This work has benefited from the equipment and framework of the COMP-HUB and COMP-R Initiatives, funded by the 'Departments of Excellence' program of the Italian Ministry for University and Research (MIUR, 2018-2022 and MUR, 2023-2027)

P28

Electrochemical genoassay on magnetic beads for ultra-sensitive detection of KRAS oncogene mutation associated with colorectal cancer in liquid biopsy

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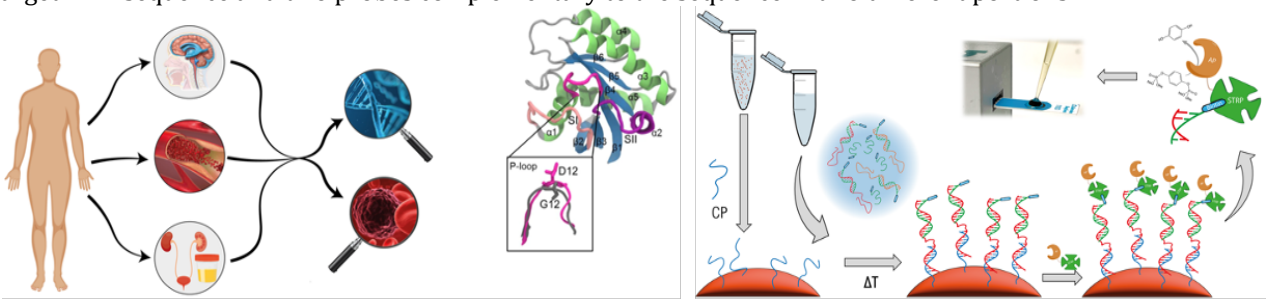
Liquid biopsy is a new diagnostic method aimed at detecting different tumour biomarkers, including cell-free DNA (cfDNA) and circulating tumour DNA (ctDNA), in biological fluids[1]. One possible application of this technology is the detection of ctDNA sequences related to single nucleotide polymorphisms in the KRAS oncogene, often correlated with the onset of colorectal cancer (CRC).
Several methodologies have been devised for the detection of ctDNA in liquid biopsy; among them, biosensing techniques guarantee a high portability of the instrumentation, which can usually be applied to point-of-care testing[2], even if discrimination of single nucleotide polymorphism (SNP) sequences is often based on modest differences of the signal output[3]. In this context, we developed an electrochemical genoassay based on PNA probes immobilized on micromagnetic beads to detect a specific KRAS mutation, namely G12D, associated with colorectal cancer. This mutation is important due to its correlation with targeted therapy and personalized medicine, as patients presenting with this mutation are resistant to anti-EGFR antibodies therapy.
The methodological approach of the genoassay is based on the formation of a "sandwich" complex between the target DNA sequence and two probes complementary to the sequence in two different portions.



In particular, two PNA Capture Probe sequences that specifically recognize wild-type and mutated sequences were used, while a DNA signalling probe, complementary to another portion of the target sequences, was used to obtain the electrochemical signal[4].
Thanks to the presence of the signalling probe, it was possible to immobilize on the geno-complex an enzyme capable of providing an amplified electrochemical signal after processing of a specific substrate.
In order to obtain the optimal level of differentiation in signal output between the complementary and non-complementary target sequences using the two capture probes, a 3^2 full-factorial design was performed, considering target concentration and incubation temperature as factors. For both capture probe sequences it was possible to obtain a reduction in signal higher than 99% between the complementary and non-complementary target sequences under optimized conditions.
Excellent limits of detection were obtained both for the capture probe complementary to G12D sequence (818 fM) and the wild-type sequence (1,8 pM) in 10-fold diluted human plasma with buffer.
The outstanding performance of this genoassay make it a valuable analytical tool for determination of the K-RAS mutation at low levels in early diagnosis of colorectal cancer.

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P29

Preliminary investigation on the combination of GC-MS and HPLC-UV/PDA methodologies for monitoring honeybee (*Apis mellifera*) metabolic and foraging status

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Pesticide exposure [1], spread of parasites and environmental and climate changes are among the reasons hypothesized for bee colonies reduction observed over the past decade [2]. However, limited data on the individual and combined impact exerted by the influence of these factor on honeybee health status are available. In the present study we investigated the potential of GC-MS and HPLC-UV/DAD approaches to furnish data useful to the description of the metabolic and foraging status of honeybees. Bees were sampled on-field and then the solvent-extractable components present in their head, thorax and abdomen analysed (a) directly by HPLC-UV/DAD and by (b) GC-MS after derivatization (BSFTA/pyridine). The results indicated the complementary capability of the two techniques to provide information on (A) bee metabolic (thorax) and (B) foraging status (abdomen) based on the selective detection and semiquantitative determination of specific bee and plant derived metabolites. In abdomen, the detected presence of plant-derived flavonoids detected by HPLC-UV/PDA, and a metabolomic profile including mono-, di- and tri-saccharides, amino acids, lipids (FA, sterols) detected by GC-MS, provide direct indications about bees' nutritional status. In parallel, metabolites detected in the thorax related to energy metabolism and protein turn-over furnish useful indications about physical performance. Taken together, the results of this preliminary study suggest the potential utility of such analytical approach for the evaluation of the health status of honeybees in different environmental conditions.

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ISBN 978-88-94952-35-3

ISBN: 978-88-94952-35-3